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(71) Applicant: **ALNYLAM PHARMACEUTICALS, INC.**
[US/US]; 675 West Kendall Street, Henri A. Termeer
Square, Cambridge, Massachusetts 02142 (US).

(72) Inventors: **ZUBER, Jeffrey**; c/o Alnylam Pharmaceu-
ticals, Inc., 675 West Kendall Street, Henri A. Ter-
meer Square, Cambridge, Massachusetts 02142 (US).
MCININCH, James D.; c/o Alnylam Pharmaceuticals,
Inc., 675 West Kendall Street, Henri A. Termeer Square,
Cambridge, Massachusetts 02142 (US). **SCHLEGEL,**
Mark K.; c/o Alnylam Pharmaceuticals, Inc., 675 West
Kendall Street, Henri A. Termeer Square, Cambridge,
Massachusetts 02142 (US). **CASTORENO, Adam**; c/o Al-
nylam Pharmaceuticals, Inc., 675 West Kendall Street, Hen-
ri A. Termeer Square, Cambridge, Massachusetts 02142
(US). **BONDURANT, Lucas**; c/o Alnylam Pharmaceu-
ticals, Inc., 675 West Kendall Street, Henri A. Termeer
Square, Cambridge, Massachusetts 02142 (US). **OZA,**
Dhaval; c/o Alnylam Pharmaceuticals, Inc., 675 West
Kendall Street, Henri A. Termeer Square, Cambridge,
Massachusetts 02142 (US).

(74) Agent: **MEIGS, Julie Broadus** et al.; Womble Bond
Dickinson (US) LLP, P.O. Box 570489, Atlanta, Georgia
30357-0037 (US).

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(54) Title: CELL DEATH-INDUCING DFFA-LIKE EFFECTOR B (CIDEB) IRNA COMPOSITIONS AND METHODS OF USE THEREOF

(57) Abstract: The invention relates to double-stranded ribonucleic acid (dsRNA) compositions targeting the CIDEB gene, as well as methods of inhibiting expression of CIDEB, and methods of treating subjects that would benefit from reduction in expression of CIDEB, such as subjects having a CIDEB-associated disease, disorder, or condition, using such dsRNA compositions.



CELL DEATH-INDUCING DFFA-LIKE EFFECTOR B (CIDEB) iRNA COMPOSITIONS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 63/239,271, filed on August 31, 2021, and claims the benefit of priority to U.S. Provisional Application No. 63/341,848, filed on May 13, 2022. The entire contents of the foregoing applications are hereby incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. The XML copy, created on August 30, 2022, is named A108868_1300WO_SL.xml and is 3,873,182 bytes in size.

BACKGROUND OF THE INVENTION

Cell death-inducing DFFA-like effector B (CIDEB), a member of the CIDE protein family, is primarily expressed in liver and small intestine. CIDEB is an endoplasmic reticulum (ER)- and lipid droplet (LD)-associated protein. Overexpression of CIDEB protein induces cell death, but the physiological function of CIDEB is more closely related to various lipid metabolic pathways, particularly the VLDL pathway.

Non-alcoholic fatty liver disease (NAFLD) is the commonest form of liver disease in all regions of the world with modern industrialized economies, including Korea and many other Asian countries. Patients usually present without symptoms or clinical features are non-specific. Instead, liver abnormalities are found incidentally by hepatic imaging, particularly ultrasonography, and/or there are raised liver enzymes (alanine aminotransferase [ALT] and gamma-glutamyltranspeptidase). The diagnosis of NAFLD requires exclusion of other disorders, particularly viral hepatitis, significant alcohol intake, and exposure to potentially hepatotoxic medications. By agreements such as the Asia-Pacific Guidelines on NAFLD, the term NAFLD is now retained for cases of fatty liver associated with metabolic complications of over-nutrition, usually with central obesity and overweight.

Non-alcoholic steatohepatitis (NASH) is considered the progressive form of nonalcoholic fatty liver disease (NAFLD) and is characterized by liver steatosis, inflammation, hepatocellular injury and different degrees of fibrosis. Adipose tissue dysfunction and the hepatic inflammatory response have a fundamental role during NASH development. Cellular and molecular response mechanisms also promote liver inflammation in the absence of a fatty liver by inducing a chronic inflammatory response that results in hepatocyte damage.

Accordingly, there is a need for improved methods of treating chronic inflammatory diseases of the liver, such as NASH, including agents that can selectively and efficiently inhibit the CIDEB gene.

BRIEF SUMMARY OF THE INVENTION

There is a need for improved methods of treating chronic inflammatory diseases of the liver, such as NASH, including agents that can selectively and efficiently inhibit the CIDEB gene. Current standards of care for subjects with chronic inflammatory diseases include lifestyle modifications (diet and exercise, cessation of smoking, drinking, etc.), steroidal and/or nonsteroidal anti-inflammatory medications, and management of associated comorbidities, *e.g.*, hypertension, hyperlipidemia, diabetes, *etc.* Once established, chronic inflammatory conditions can maintain a self-perpetuating cycle of inflammation, tissue damage, release of proinflammatory damage-associated molecular patterns (DAMPs) from injured cells, and cytokine release leading to further inflammation. Abrogation of liver inflammation could be achieved by exploiting active, physiological pro-resolving mechanisms instead of the classical passive blockade of pro-inflammatory mediators. (Schuster *et al.*, *Nature Reviews Gastroenterology & Hepatology*, volume 15, pages 349–364, 2018).

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a cell death-inducing DFFA-like effector b (CIDEB) gene. The CIDEB gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The present invention also provides methods of using the iRNA compositions of the invention for inhibiting the expression of a CIDEB gene and/or for treating a subject who would benefit from inhibiting or reducing the expression of a CIDEB gene, *e.g.*, a subject suffering or prone to suffering from a CIDEB-associated disease, for example, a chronic inflammatory disease.

Accordingly, in one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of cell death-inducing DFFA-like effector b (CIDEB) in a cell. The dsRNA agent includes a sense strand and an antisense strand, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO: 1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO: 2. In some embodiments, the dsRNA agent includes a sense strand and an antisense strand, wherein the sense strand comprises at least 15 contiguous nucleotides from the nucleotide sequence of SEQ ID NO: 1 and the antisense strand comprises at least 15 contiguous nucleotides from the nucleotide sequence of SEQ ID NO: 2.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of cell death-inducing DFFA-like effector b (CIDEB) in a cell. The dsRNA agent includes a sense strand and an antisense strand forming a double stranded region, wherein said antisense strand comprises a region of complementarity to an mRNA encoding CIDEB which comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from any one of the antisense sequences listed in Tables 3-6. In some embodiments, the dsRNA agent includes a sense strand and an

antisense strand forming a double stranded region, wherein said antisense strand comprises a region of complementarity to an mRNA encoding CIDEb which comprises at least 15 contiguous nucleotides from any one of the antisense sequences listed in Tables 3-6.

In one embodiment, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of cell death-inducing DFFA-like effector b (CIDEb), wherein the dsRNA agent comprises (a) an antisense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700555 and a sense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700555; (b) an antisense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700821 and a sense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700821; (c) an antisense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700369 and a sense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700369; (d) an antisense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1699976 and a sense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1699976; (e) an antisense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700374 and a sense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700374; (f) an antisense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700314 and a sense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700314; (g) an antisense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700376 and a sense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700376; (h) an antisense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1699964 and a sense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide

sequence of duplex AD-1699964; or (i) an antisense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700556 and a sense strand comprising a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700556.

5 In another embodiment, the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from any one of nucleotides 29-51, 67-89, 154-176, 163-185, 173-195, 184-206, 196-218, 206-228, 257-279, 270-292, 446-468, 459-481, 468-490, 518-540, 530-552, 641-663, 687-709, 702-724, 711-733, 727-749, 758-780, 769-791, 781-803, 790-812, 807-829, 839-861, 850-872, 874-896, 907-929, 917-939, 958-980, 974-996, 983-1005, 999-1021, 1009-
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5 region of complementarity comprises at least 15 contiguous nucleotides from any one of nucleotides 29-51, 67-89, 154-176, 163-185, 173-195, 184-206, 196-218, 206-228, 257-279, 270-292, 446-468, 459-481, 468-490, 518-540, 530-552, 641-663, 687-709, 702-724, 711-733, 727-749, 758-780, 769-791, 781-803, 790-812, 807-829, 839-861, 850-872, 874-896, 907-929, 917-939, 958-980, 974-996, 983-1005, 999-1021, 1009-1031, 1018-1040, 1027-1049, 1036-1058, 1045-1067, 1054-1076, 1080-1102, 1089-1111,
10 1098-1120, 1108-1130, 1140-1162, 1156-1178, 1174-1196, 1183-1205, 1192-1214, 1205-1227, 1214-1236, 1225-1247, 1254-1276, 1263-1285, 1273-1295, 1282-1304, 1292-1314, 1316-1338, 1337-1359, 1350-1372, 1364-1386, 1375-1397, 1408-1430, 1417-1439, 1429-1451, 1454-147, 1478-1500, 1487-1509, 1496-1518, 1507-1529, 1519-1541, 1542-1564, 1552-1574, 1562-1584, 1573-1595, 1585-1607, 1597-1619, 1607-1629, 1623-1645, 1633-1655, 1642-1664, 1651-1673, 1668-1690, 1677-1699, 1691-1713, 1700-1722, 1712-1734, 1749-1771, 1764-1786, 1773-1795, 1784-1806, 1796-1818, 1807-1829,
15 1824-1846, 1833-1855, 1847-1869, 1856-1878, 1865-1887, 1881-1903, 1896-1918, 1905-1927, 1921-1943, 1938-1960, 1948-1970, 1961-1983, 1970-1992, 1994-2016, 2008-2030, 2017-2039, 2056-2078, 2066-2088, 2075-2097, 2087-2109, 2096-2118, 2106-2128, 2116-2138, 2129-2151, 2176-2198, 2185-2207, 2196-2218, 2207-2229, 2220-2242, 2236-2258, 2247-2269, 2256-2278, 2265-2287, 2274-2296, 2299-2321, 2309-2331, 2318-2340, 2349-2371, 2371-2393, 2382-2404, 2391-2413, 2401-2423, 1267-1289, 1270-1292, 1271-1293, 1272-1294, 1273-1295, 1274-1296, 1275-1297, 1276-1298, 1278-1300, 1285-1307, 1294-1316, 1295-1317, 1327-1349, 1330-1352, 1371-1393, 1372-1394, 1374-1396, 1407-1429, 1410-1432, 1413-1435, 1414-1436, 1415-1437, 1416-1438, 1419-1441, 1420-1442, 1421-1443, 1422-1444, 1425-1447, 1426-1448, 1427-1449, 1428-1450, 1429-1451, 1430-1452, 1431-1453, 1432-25 1454, 1433-1455, 1478-1500, 1498-1520, 1500-1522, 1501-1523, 1502-1524, 1503-1525, 1504-1526, 1545-1567, 1548-1570, 1549-1571, 1550-1572, 1551-1573, 1559-1581, 1560-1582, 1562-1584, 1565-1587, 1567-1589, 1568-1590, 1569-1591, 1572-1594, 1577-1599, 1580-1602, 1581-1603, 1582-1604, 1583-1605, 1584-1606, 1589-1611, 1590-1612, 1593-1615, 1616-1638, 1617-1639, 1624-1646, 1626-1648, 1627-1649, 1628-1650, 1634-1656, 1635-1657, 1648-1670, 1655-1677, 1656-1678, 1657-1679, 1658-1680, 1659-1681, 1661-1683, 1681-1703, 1710-1732, 1711-1733, 1712-1734, 1713-1735, 1716-30 1738, 1717-1739, 1718-1740, 1720-1742, 1744-1766, 1751-1773, 1752-1774, 1775-1797, 1781-1803, 1784-1806, 1786-1808, 1787-1809, 1788-1810, 1789-1811, 1790-1812, 1795-1817, 1796-1818, 1797-1819, 1799-1821, 1800-1822, 1801-1823, 1808-1830, 1811-1833, 1816-1838, 1822-1844, 1824-1846, 1825-1847, 1826-1848, 1827-1849, 1828-1850, 1829-1851, 1830-1852, 1831-1853, 1837-1859, 1838-35 1860, 1840-1862, 1841-1863, 1842-1864, 1843-1865, 1844-1866, 1846-1868, 1847-1869, 1848-1870,

1850-1872, 1855-1877, 1856-1878, 1857-1879, 1858-1880, 1859-1881, 1860-1882, 1880-1902, 1882-1904, 1883-1905, 1885-1907, 1886-1908, 1894-1916, 1895-1917, 1896-1918, 1897-1919, 1898-1920, 1899-1921, 1900-1922, 1911-1933, 1933-1955, 1934-1956, 1936-1958, 1937-1959, 1940-1962, 1945-1967, 1946-1968, 1948-1970, 1949-1971, 1951-1973, 1954-1976, 1957-1979, 1958-1980, 1959-1981, 5 1960-1982, 1961-1983, 1962-1984, 2011-2033, 2013-2035, 2014-2036, 2016-2038, 2074-2096, 2076-2098, 2082-2104, 2085-2107, 2086-2108, 2087-2109, 2088-2110, 2089-2111, 2090-2112, 2092-2114, 2095-2117, 2098-2120, 2105-2127, 2107-2129, 2108-2130, 2110-2132, 2112-2134, 2114-2136, 2192-2214, 2239-2261, 2240-2262, 2249-2271, 2250-2272, 2253-2275, 2300-2322, 2346-2368, 2347-2369, 2348-2370, 2432-2454, 2433-2455, 2434-2456, 1267-1289, 1276-1298, 1277-1299, 1279-1301, 1283-10 1305, 1284-1306, 1285-1307, 1286-1308, 1292-1314, 1295-1317, 1319-1341, 1328-1350, 1329-1351, 1330-1352, 1331-1353, 1332-1354, 1340-1362, 1341-1363, 1342-1364, 1343-1365, 1344-1366, 1345-1367, 1346-1368, 1368-1390, 1371-1393, 1373-1395, 1375-1397, 1408-1430, 1417-1439, 1418-1440, 1419-1441, 1423-1445, 1424-1446, 1430-1452, 1431-1453, 1437-1459, 1443-1465, 1478-1500, 1503-1525, 1512-1534, 1544-1566, 1545-1567, 1546-1568, 1547-1569, 1552-1574, 1553-1575, 1560-1582, 15 1561-1583, 1563-1585, 1566-1588, 1567-1589, 1570-1592, 1571-1593, 1572-1594, 1573-1595, 1574-1596, 1578-1600, 1579-1601, 1580-1602, 1584-1606, 1585-1607, 1586-1608, 1593-1615, 1595-1617, 1599-1621, 1600-1622, 1603-1625, 1609-1631, 1611-1633, 1612-1634, 1613-1635, 1614-1636, 1616-1638, 1618-1640, 1619-1641, 1620-1642, 1621-1643, 1622-1644, 1623-1645, 1625-1647, 1629-1651, 1632-1654, 1633-1655, 1635-1657, 1640-1662, 1645-1667, 1647-1669, 1651-1673, 1656-1678, 1657-20 1679, 1660-1682, 1680-1702, 1704-1726, 1705-1727, 1707-1729, 1709-1731, 1713-1735, 1714-1736, 1715-1737, 1716-1738, 1719-1741, 1720-1742, 1749-1771, 1773-1795, 1774-1796, 1775-1797, 1776-1798, 1778-1800, 1782-1804, 1783-1805, 1784-1806, 1785-1807, 1791-1813, 1792-1814, 1793-1815, 1805-1827, 1809-1831, 1810-1832, 1812-1834, 1813-1835, 1815-1837, 1817-1839, 1818-1840, 1819-1841, 1826-1848, 1833-1855, 1834-1856, 1836-1858, 1838-1860, 1839-1861, 1841-1863, 1844-1866, 25 1846-1868, 1847-1869, 1851-1873, 1852-1874, 1853-1875, 1854-1876, 1856-1878, 1860-1882, 1861-1883, 1880-1902, 1881-1903, 1882-1904, 1884-1906, 1887-1909, 1888-1910, 1889-1911, 1893-1915, 1900-1922, 1902-1924, 1910-1932, 1912-1934, 1917-1939, 1936-1958, 1941-1963, 1942-1964, 1944-1966, 1945-1967, 1949-1971, 1955-1977, 1962-1984, 2011-2033, 2015-2037, 2016-2038, 2074-2096, 2075-2097, 2076-2098, 2083-2105, 2091-2113, 2093-2115, 2094-2116, 2095-2117, 2096-2118, 2102-30 2124, 2103-2125, 2106-2128, 2109-2131, 2111-2133, 2112-2134, 2113-2135, 2115-2137, 2117-2139, 2241-2263, 2250-2272, 2253-2275, 2300-2322, 2301-2323, 2343-2365, 2347-2369, 2349-2371, 1796-1818, 1270-1292, 1624-1646, 1795-1817, 1822-1844, 1847-1869, 1582-1604, 1582-1604, 1582-1604, 1570-1592, 2110-2132, 1954-1976, 2013-2035, 1840-1862, 2114-2136, 1565-1587, 1808-1830, 2249-2271, 1614-1636, 1655-1677, 1581-1603, 1717-1739, 1717-1739, 1717-1739, 1883-1905, 1894-1916, 35 1957-1979, 1957-1979, 1957-1979, 1786-1808, 1681-1703, 1589-1611, 2098-2120, 1327-1349, 2432-

2454, 1374-1396, 1911-1933, 1372-1394, 1816-1838, 2240-2262, 1577-1599, 2105-2127, 2092-2114, 1951-1973, 2346-2368, 1781-1803, 2014-2036, 1410-1432, 1940-1962, 1718-1740, 1859-1881, 1744-1766, 1787-1809, 1787-1809, 1787-1809, 1855-1877, or 2082-2104 of SEQ ID NO: 1.

In one embodiment, the dsRNA agent comprises at least one modified nucleotide.

5 In one embodiment, substantially all of the nucleotides of the sense strand comprise a modification. In another embodiment, substantially all of the nucleotides of the antisense strand comprise a modification. In yet another embodiment, substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification.

10 In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell. The dsRNA agent includes a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:2, wherein
15 substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the sense strand is conjugated to a ligand attached at the 3'-terminus. In some embodiments, the dsRNA agent includes a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15 contiguous nucleotides
20 from the nucleotide sequence of SEQ ID NO:2, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the sense strand is conjugated to a ligand attached at the 3'-terminus.

In one embodiment, all of the nucleotides of the sense strand comprise a modification. In another embodiment, all of the nucleotides of the antisense strand comprise a modification. In yet another
25 embodiment, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In one embodiment, at least one of said modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a
30 conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide
35 comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide

comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a glycol modified nucleotide, and a 2-O-(N-methylacetamide) modified nucleotide, and combinations thereof.

In one embodiment, the nucleotide modifications are 2'-O-methyl and/or 2'-fluoro modifications.

The region of complementarity may be at least 17 nucleotides in length; 19 to 30 nucleotides in length; 19-25 nucleotides in length; or 21 to 23 nucleotides in length.

Each strand may be no more than 30 nucleotides in length, *e.g.*, each strand is independently 19-30 nucleotides in length; each strand is independently 19-25 nucleotides in length; each strand is independently 21-23 nucleotides in length.

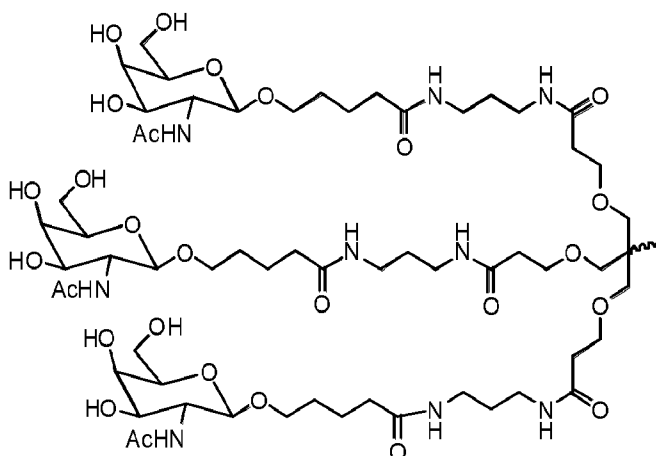
The dsRNA may include at least one strand that comprises a 3' overhang of at least 1 nucleotide; or at least one strand that comprises a 3' overhang of at least 2 nucleotides.

In some embodiment, the dsRNA agent further comprises a ligand.

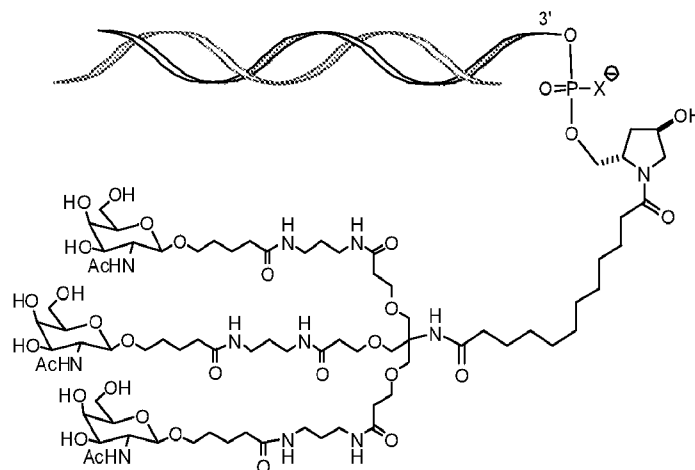
In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

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

In one embodiment, the ligand is



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

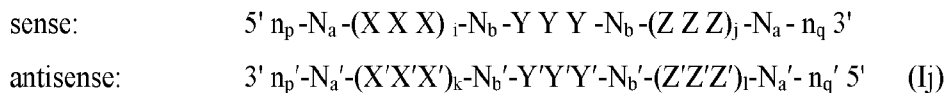


and, wherein X is O or S.

In one embodiment, the X is O.

In one embodiment, the region of complementarity comprises any one of the antisense sequences in Tables 3-6.

In one aspect, the present invention provides a double stranded for inhibiting expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell. The dsRNA agent includes a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CIDEb, wherein each strand is about 14 to about 30 nucleotides in length, wherein said dsRNA agent is represented by formula (Ij):



wherein:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

each n_p , n_p' , n_q , and n_q' , each of which may or may not be present, independently represents an overhang nucleotide;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

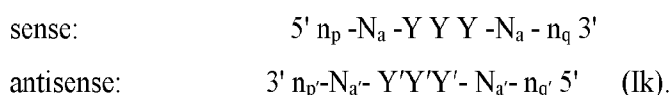
wherein the sense strand is conjugated to at least one ligand.

In one embodiment, i is 0; j is 0; i is 1; j is 1; both i and j are 0; or both i and j are 1. In another embodiment, k is 0; l is 0; k is 1; l is 1; both k and l are 0; or both k and l are 1.

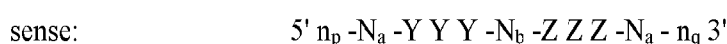
In one embodiment, XXX is complementary to X'X'X', YYY is complementary to Y'Y'Y', and ZZZ is complementary to Z'Z'Z'.

In one embodiment, the YYY motif occurs at or near the cleavage site of the sense strand, *e.g.*, the Y'Y'Y' motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end.

In one embodiment, formula (Ij) is represented by formula (Ik):



In another embodiment, formula (Ij) is represented by formula (Il):



antisense: $3' n_p - N_a - Y'Y'Y' - N_b - Z'Z'Z' - N_a - n_q 5'$ (II)

wherein each N_b and N_b' independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides.

In yet another embodiment, formula (Ij) is represented by formula (Im):

sense: $5' n_p - N_a - X X X - N_b - Y Y Y - N_a - n_q 3'$

antisense: $3' n_p - N_a - X'X'X' - N_b - Y'Y'Y' - N_a - n_q 5'$ (Im)

wherein each N_b and N_b' independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides.

In another embodiment, formula (Ij) is represented by formula (In):

sense: $5' n_p - N_a - X X X - N_b - Y Y Y - N_b - Z Z Z - N_a - n_q 3'$

antisense: $3' n_p - N_a - X'X'X' - N_b - Y'Y'Y' - N_b - Z'Z'Z' - N_a - n_q 5'$ (In)

wherein each N_b and N_b' independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides and each N_a and N_a' independently represents an oligonucleotide sequence comprising 2-10 modified nucleotides.

The region of complementarity may be at least 17 nucleotides in length; 19 to 30 nucleotides in length; 19-25 nucleotides in length; or 21 to 23 nucleotides in length.

Each strand may be no more than 30 nucleotides in length, *e.g.*, each strand is independently 19-30 nucleotides in length.

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

In one embodiment, the modifications on the nucleotides are 2'-O-methyl or 2'-fluoro modifications.

In one embodiment, the Y' is a 2'-O-methyl or 2'-fluoro modified nucleotide.

In one embodiment, at least one strand of the dsRNA agent may comprise a 3' overhang of at least 1 nucleotide; or a 3' overhang of at least 2 nucleotides.

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

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand. In one embodiment, the strand is the antisense strand. In another embodiment, the strand is the sense strand.

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

In one embodiment, the strand is the antisense strand. In another embodiment, the strand is the sense strand.

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

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

5 In one embodiment, $p' > 0$. In another embodiment, $p' = 2$.

In one embodiment, $q' = 0$, $p = 0$, $q = 0$, and p' overhang nucleotides are complementary to the target mRNA. In another embodiment, $q' = 0$, $p = 0$, $q = 0$, and p' overhang nucleotides are non-complementary to the target mRNA.

10 In one embodiment, the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

In one embodiment, at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage. In another embodiment, wherein all n_p' are linked to neighboring nucleotides via phosphorothioate linkages.

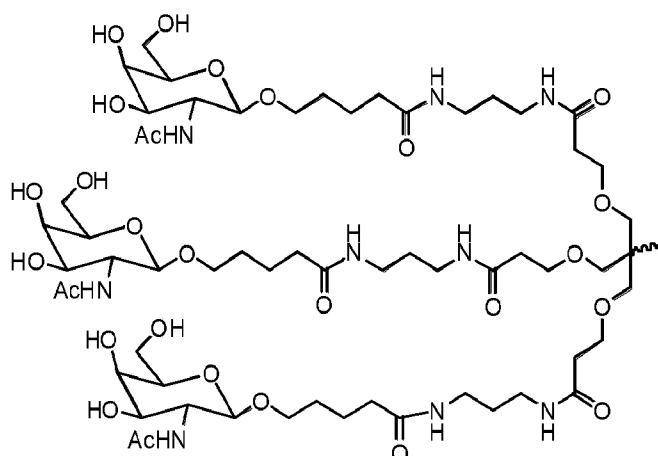
15 In one embodiment, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

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

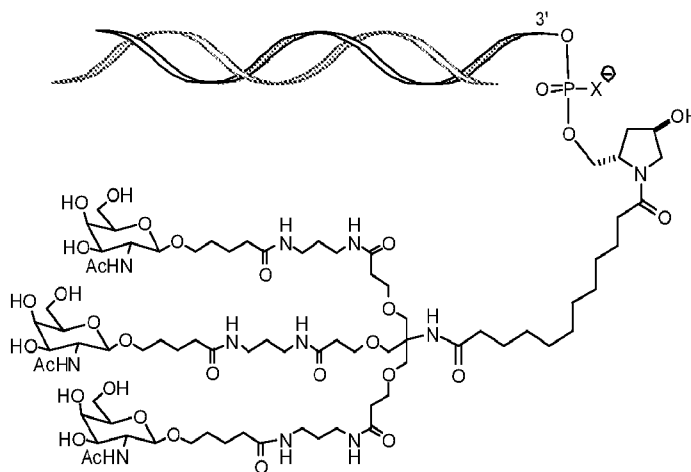
In one embodiment, the ligand is one or more N-acetylgalactosamine (GalNAc) derivatives attached through a monovalent, bivalent, or trivalent branched linker.

In one embodiment, the ligand is

20



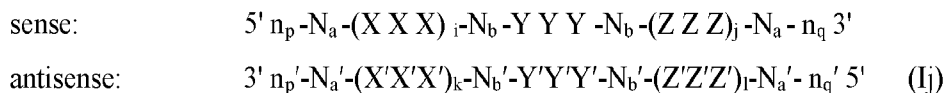
In one embodiment, the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

In one embodiment, the X is O.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell. The dsRNA agent includes a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CIDEb, wherein each strand is about 14 to about 30 nucleotides in length, wherein the dsRNA agent is represented by formula (Ij):



wherein:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and $N_{a'}$ independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and $N_{b'}$ independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

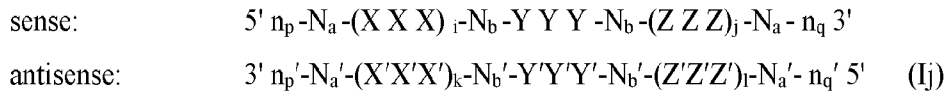
each n_p , $n_{p'}$, n_q , and $n_{q'}$, each of which may or may not be present independently represents an overhang nucleotide;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on $N_{b'}$ differ from the modification on Y'; and

wherein the sense strand is conjugated to at least one ligand.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell. The dsRNA agent includes a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CIDEb, wherein each strand is about 14 to about 30 nucleotides in length, wherein the dsRNA agent is represented by formula (Ij):



wherein:

i, j, k, and l are each independently 0 or 1;

each n_p , n_q , and n_q' , each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q' are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides

which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

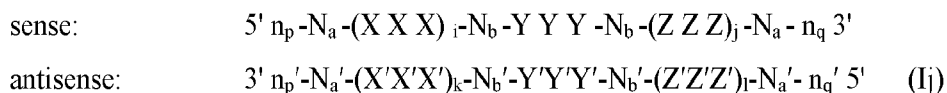
each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

wherein the sense strand is conjugated to at least one ligand.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell. The dsRNA agent includes a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CIDEb, wherein each strand is about 14 to about 30 nucleotides in length, wherein the dsRNA agent is represented by formula (Ij):



wherein:

i, j, k, and l are each independently 0 or 1;

each n_p , n_q , and n_q' , each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q' are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two
5 differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-
10 fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

15 In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell. The dsRNA agent includes a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CIDEb, wherein each strand is about 14 to about 30 nucleotides in length, wherein the dsRNA agent is represented by formula (Ij):

20 sense: $5' n_p - N_a - (X X X)_i - N_b - Y Y Y - N_b - (Z Z Z)_j - N_a - n_q 3'$
antisense: $3' n_p' - N_a' - (X'X'X')_k - N_b' - Y'Y'Y' - N_b' - (Z'Z'Z')_l - N_a' - n_q' 5'$ (Ij)

wherein:

i, j, k, and l are each independently 0 or 1;

each n_p , n_q , and n_q' , each of which may or may not be present, independently represents an overhang
25 nucleotide;

p, q, and q' are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two
30 differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-
35 fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y';

wherein the sense strand comprises at least one phosphorothioate linkage; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more

5 GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell. The dsRNA agent includes a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CIDEb, wherein each strand is about 14 to about 30 nucleotides
10 in length, wherein the dsRNA agent is represented by formula (Ij):

sense: $5' n_p - N_a - Y Y Y - N_a - n_q 3'$

antisense: $3' n_p' - N_a' - Y'Y'Y' - N_a' - n_q' 5'$ (Ik)

wherein:

each n_p , n_q , and n_q' , each of which may or may not be present, independently represents an overhang
15 nucleotide;

p , q , and q' are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two
20 differently modified nucleotides;

YYY and Y'Y'Y' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl and/or 2'-fluoro modifications;

wherein the sense strand comprises at least one phosphorothioate linkage; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more

25 GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell. The dsRNA agent includes a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the
30 nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:2, wherein substantially all of the nucleotides of the sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the sense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus, wherein substantially all of the nucleotides
35 of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl

modification and a 2'-fluoro modification, wherein the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and wherein the sense strand is conjugated to one or more GalNAc derivatives attached through a monovalent, bivalent or trivalent branched linker at the 3'-terminus. In some embodiments, the dsRNA agent
5 includes a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15 contiguous nucleotides from the nucleotide sequence of SEQ ID NO:2, wherein substantially all of the nucleotides of the sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the sense strand comprises
10 two phosphorothioate internucleotide linkages at the 5'-terminus, wherein substantially all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and wherein the sense strand is conjugated to one or more GalNAc derivatives attached through a
15 monovalent, bivalent or trivalent branched linker at the 3'-terminus.

In one embodiment, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides.

In one embodiment, the region of complementarity comprises any one of the antisense sequences listed in Tables 3-6.

20 In one embodiment, the sense strand and the antisense strand comprise nucleotide sequences selected from the group consisting of the nucleotide sequences of any one of the agents listed in Tables 3-6.

In various embodiments of the aforementioned dsRNA agents, the dsRNA agent targets a hotspot region of an mRNA encoding CIDEB.

25 In another aspect, the present invention provides a dsRNA agent that targets a hotspot region of a cell death-inducing DFFA-like effector B (CIDEB) mRNA.

The present invention also provides cells, vectors, and pharmaceutical compositions which include any of the dsRNA agents of the invention. The dsRNA agents may be formulated in an unbuffered solution, *e.g.*, saline or water, or in a buffered solution, *e.g.*, a solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof. In one embodiment, the buffered solution is phosphate buffered
30 saline (PBS).

In one aspect, the present invention provides a method of inhibiting cell death-inducing DFFA-like effector b (CIDEB) expression in a cell. The method includes contacting the cell with a dsRNA agent or a pharmaceutical composition of the invention, thereby inhibiting expression of CIDEB in the cell.

The cell may be within a subject, such as a human subject.

In one embodiment, the CIDEB expression is inhibited by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or to below the level of detection of CIDEB expression.

In one embodiment, the human subject suffers from a CIDEB-associated disease, disorder, or condition. In one embodiment, the CIDEB-associated disease, disorder, or condition is a chronic inflammatory disease, such as a chronic inflammatory disease of the liver and other tissues. In one embodiment, the chronic inflammatory disease is chronic inflammatory liver disease. In one embodiment, the chronic inflammatory liver disease is selected from the group consisting of accumulation of fat in the liver, inflammation of the liver, liver fibrosis, fatty liver disease (steatosis), nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD) and cirrhosis of the liver.

In one aspect, the present invention provides a method of inhibiting the expression of CIDEB in a subject. The methods include administering to the subject a therapeutically effective amount of a dsRNA agent or a pharmaceutical composition of the invention, thereby inhibiting the expression of CIDEB in the subject.

In another aspect, the present invention provides a method of treating a subject suffering from a CIDEB-associated disease, disorder, or condition. The method includes administering to the subject a therapeutically effective amount of a dsRNA agent or a pharmaceutical composition of the invention, thereby treating the subject suffering from a CIDEB-associated disease, disorder, or condition.

In another aspect, the present invention provides a method of preventing at least one symptom in a subject having a disease, disorder or condition that would benefit from reduction in expression of a CIDEB gene. The method includes administering to the subject a prophylactically effective amount of the agent of a dsRNA agent or a pharmaceutical composition of the invention, thereby preventing at least one symptom in a subject having a disease, disorder or condition that would benefit from reduction in expression of a CIDEB gene.

In another aspect, the present invention provides a method of reducing the risk of developing chronic liver disease in a subject having steatosis. The method includes administering to the subject a therapeutically effective amount of a dsRNA agent or a pharmaceutical composition of the invention, thereby reducing the risk of developing chronic liver disease in the subject having steatosis.

In one aspect, the present invention provides a method of inhibiting the accumulation of lipid droplets in the liver of a subject suffering from a CIDEB-associated disease, disorder, or condition. The method includes administering to the subject a therapeutically effective amount of a dsRNA agent or a pharmaceutical composition of the invention, and a dsRNA agent targeting a CIDEB gene or a pharmaceutical composition comprising a dsRNA agent targeting a CIDEB gene, thereby inhibiting the accumulation of fat in the liver of the subject suffering from a CIDEB-associated disease, disorder, or condition.

In another aspect, the present invention provides a method of treating a subject suffering from a CIDEB-associated disease, disorder, or condition. The method includes administering to the subject a

therapeutically effective amount of a dsRNA agent or a pharmaceutical composition of the invention, and a dsRNA agent targeting a CIDEB gene or a pharmaceutical composition comprising a dsRNA agent targeting a CIDEB gene, thereby treating the subject suffering from a CIDEB-associated disease, disorder, or condition.

5 In another aspect, the present invention provides a method of preventing at least one symptom in a subject having a disease, disorder or condition that would benefit from reduction in expression of a CIDEB gene. The method includes administering to the subject a therapeutically effective amount of a dsRNA agent or a pharmaceutical composition of the invention, and a dsRNA agent targeting a CIDEB gene or a pharmaceutical composition comprising a dsRNA agent targeting a CIDEB gene, thereby preventing at least one symptom in a subject having a disease, disorder or condition that would benefit from reduction in
10 expression of a CIDEB gene.

In another aspect, the present invention provides a method of reducing the risk of developing chronic liver disease in a subject having steatosis. The method includes administering to the subject a therapeutically effective amount of a dsRNA agent or a pharmaceutical composition of the invention, and a dsRNA agent targeting a CIDEB gene or a pharmaceutical composition comprising a dsRNA agent targeting a CIDEB gene,
15 thereby reducing the risk of developing chronic liver disease in the subject having steatosis.

In another aspect, the present invention provides a method of inhibiting the progression of steatosis to steatohepatitis in a subject suffering from steatosis. The method includes administering to the subject a therapeutically effective amount of a dsRNA agent or a pharmaceutical composition of the invention, and a dsRNA agent targeting a CIDEB gene or a pharmaceutical composition comprising a dsRNA agent targeting a
20 CIDEB gene, thereby inhibiting the progression of steatosis to steatohepatitis in the subject.

In one embodiment, the administration of the dsRNA agent or the pharmaceutical composition to the subject causes a decrease in CIDEB protein activity, *e.g.*, a decrease in the interaction of CIDEB with ApoB and/or a decrease in lipid maturation in liver; a decrease in CIDEB protein accumulation, a decrease in CIDEBenzymatic activity, a decrease in CIDEBprotein accumulation, and/or a decrease in accumulation of fat
25 and/or expansion of lipid droplets in the liver of a subject.

In one embodiment, the CIDEB-associated disease, disorder, or condition is a chronic inflammatory disease.

In one embodiment, the chronic inflammatory disease is chronic inflammatory liver disease.

30 In one embodiment, the chronic inflammatory liver disease is selected from the group consisting of accumulation of fat in the liver, inflammation of the liver, liver fibrosis, fatty liver disease (steatosis), nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD) and cirrhosis of the liver.

In one embodiment, the chronic inflammatory liver disease is nonalcoholic steatohepatitis (NASH).

In one embodiment, the subject is obese.

35 In one embodiment, the methods and uses of the invention further include administering an additional therapeutic to the subject.

In one embodiment, the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg.

The agent may be administered to the subject intravenously, intramuscularly, or subcutaneously. In one embodiment, the agent is administered to the subject subcutaneously.

5 In one embodiment, the methods and uses of the invention further include determining, the level of CIDEB in the subject.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of cell death-inducing DFFA-like effector b (CIDEB) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises a nucleotide sequence of any one of the agents in Tables 3-6 and the antisense strand
10 comprises a nucleotide sequence of any one of the agents in Tables 3-6, wherein substantially all of the nucleotide of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the dsRNA agent is conjugated to a ligand.

15 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts the qPCR results for CIDEB mRNA in a mouse *in vivo* single dose (3 mg/kg) study using exemplary human CIDEB dsRNA duplexes. The results are presented as the percent mRNA remaining normalized to PBS.

Fig. 2 depicts the qPCR results for CIDEB mRNA in a mouse *in vivo* multi-dose (1.5 mg/kg and
20 0.75 mg/kg) study using exemplary human CIDEB dsRNA duplexes. The results are presented as the percent mRNA remaining normalized to PBS.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides iRNA compositions, which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a CIDEB gene. The CIDEB gene may be within a cell, *e.g.*,
25 a cell within a subject, such as a human. The present invention also provides methods of using the iRNA compositions of the invention for inhibiting the expression of a CIDEB gene, and for treating a subject who would benefit from inhibiting or reducing the expression of a CIDEB gene, *e.g.*, a subject that would benefit from a reduction in inflammation, *e.g.*, a subject suffering or prone to suffering from a CIDEB-associated disease disorder, or condition, such as a subject suffering or prone to suffering from chronic inflammatory
30 diseases of the liver and other tissues, *e.g.*, a subject suffering from chronic inflammatory liver disease, such as liver fibrosis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), alcoholic steatohepatitis (ASH), alcoholic liver diseases (ALD), cirrhosis of the liver, HCV-associated cirrhosis, drug induced liver injury, hepatocellular necrosis, insulin insensitivity and diabetes.

The iRNAs of the invention targeting CIDEB may include an RNA strand (the antisense strand) having a region which is about 30 nucleotides or less in length, *e.g.*, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of a CIDEB gene.

In some embodiments, one or both of the strands of the double stranded RNAi agents of the invention is up to 66 nucleotides in length, *e.g.*, 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length, with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA transcript of a CIDEB gene. In some embodiments, such iRNA agents having longer length antisense strands may include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

The use of the iRNA agents described herein enables the targeted degradation of mRNAs of a CIDEB gene in mammals.

Very low dosages of the iRNAs, in particular, can specifically and efficiently mediate RNA interference (RNAi), resulting in significant inhibition of expression of a CIDEB gene. Thus, methods and compositions including these iRNAs are useful for treating a subject who would benefit from inhibiting or reducing the expression of a CIDEB gene, *e.g.*, a subject that would benefit from a reduction of inflammation, *e.g.*, a subject suffering or prone to suffering from a CIDEB-associated disease disorder, or condition, such as a subject suffering or prone to suffering from chronic inflammatory diseases of the liver and other tissues, *e.g.*, a subject suffering from chronic inflammatory liver disease, such as liver fibrosis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), alcoholic steatohepatitis (ASH), alcoholic liver diseases (ALD), cirrhosis of the liver, HCV-associated cirrhosis, drug induced liver injury, hepatocellular necrosis, insulin insensitivity and diabetes..

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of a CIDEB gene, as well as compositions and methods for treating subjects having diseases and disorders that would benefit from inhibition and/or reduction of the expression of this gene.

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element, *e.g.*, a plurality of elements.

The term “including” is used herein to mean, and is used interchangeably with, the phrase “including but not limited to”.

The term “or” is used herein to mean, and is used interchangeably with, the term “and/or,” unless context clearly indicates otherwise.

The term “about” is used herein to mean within the typical ranges of tolerances in the art. For example, “about” can be understood as about 2 standard deviations from the mean. In certain embodiments, about means $\pm 10\%$. In certain embodiments, about means $\pm 5\%$. When about is present before a series of numbers or a range, it is understood that “about” can modify each of the numbers in the series or range.

The term “CIDE_B,” also known as “cell death-inducing DFFA-like effector b”, “Cell Death Activator CIDE-B” or “cell death-inducing DFF45-like effector B”, refers to the well-known gene encoding a CIDE_B protein from any vertebrate or mammalian source, including, but not limited to, human, bovine, chicken, rodent, mouse, rat, porcine, ovine, primate, monkey, and guinea pig, unless specified otherwise.

The term also refers to fragments and variants of native CIDE_B that maintain at least one *in vivo* or *in vitro* activity of a native CIDE_B.

CIDE_B, a member of the CIDE protein family, is primarily expressed in liver tissues as well as in the small intestine, for example, the jejunum and ileum sections of the small intestine (Zhang *et al.*, *Lipid Res.*;55(7):1279-87; 2014). CIDE_B is an endoplasmic reticulum (ER)- and lipid droplet (LD)-associated protein. As a CIDE family member, overexpression of CIDE_B protein induces cell death, but the physiological function of CIDE_B is more closely related to various lipid metabolic pathways, particularly the VLDL pathway. For instance, CIDE_B mediates VLDL lipidation and maturation by interacting with ApoB; CIDE_B is also required for the biogenesis of VLDL transport vesicles and for chylomicron lipidation in the small intestine. CIDE_B mutant mice displayed significantly increased insulin sensitivity and enhanced rate of whole-body metabolism and hepatic fatty acid oxidation (Li *et al.*, *Diabetes*. 56(10):2523-32. 2007). Therefore, CIDE_B may represent a new therapeutic target for the treatment of obesity, diabetes, and liver steatosis (Li *et al.*, *Diabetes*. 56(10):2523-32. 2007). CIDE_B is transcriptionally regulated by hepatocyte nuclear factor 4 α (HNF4 α), the most abundant transcription factor in the liver; HNF4 α is crucial for VLDL-mediated lipid transport and participates in HCV assembly/release. As a key transcriptional coactivator of HNF4 α , peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) also regulates HCV production and PGC-1 α stimulates VLDL assembly in a CIDE_B-dependent manner (Cai *et al.*, *Scientific Reports*, volume 6, Article number: 27778, 2016).

CIDE_B is required for HCV entry into hepatocytes and HCV assembly (Xu *et al.*, *J Virol.*, 88, 8433–8444, 2014). CIDE_B interacts with the HCV NS5A protein and regulates the association of HCV particles

with ApoE. CIDEB also regulates the post-entry stages of the dengue virus (DENV) lifecycle (Cai *et al.*, *Scientific Reports*, volume 6, Article number: 27778, 2016).

Exemplary nucleotide and amino acid sequences of CIDEB can be found, for example, at GenBank Accession No. NM_001393338.1 (SEQ ID NO: 1; reverse complement SEQ ID NO: 2) for Homo sapiens.

Additional examples of CIDEB mRNA sequences are readily available using publicly available databases, *e.g.*, GenBank, UniProt, and OMIM.

Further information on CIDEB is provided, for example in the NCBI Gene database at <http://www.ncbi.nlm.nih.gov/gene/27141>.

In some embodiments, the iRNAs that are substantially complementary to a region of a mouse or rat CIDEB mRNA cross-react with human CIDEB mRNA and represent potential candidates for human targeting.

The term “CIDEB” as used herein also refers to a particular polypeptide expressed in a cell by naturally occurring DNA sequence variations of the CIDEB gene, such as a single nucleotide polymorphism in the CIDEB gene. Numerous Single Nucleotide Polymorphisms (SNPs) within the CIDEB gene have been identified and may be found at, for example, NCBI dbSNP (see, *e.g.*, www.ncbi.nlm.nih.gov/snp).

As used herein, “target sequence” refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a CIDEB gene, including mRNA that is a product of RNA processing of a primary transcription product. In one embodiment, the target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a CIDEB gene.

The target sequence of a CIDEB gene may be from about 9-36 nucleotides in length, *e.g.*, about 15-30 nucleotides in length. For example, the target sequence can be from about 15-30 nucleotides, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

As used herein, the term “strand comprising a sequence” refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

“G,” “C,” “A,” “T” and “U” each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine and uracil as a base, respectively. However, it will be understood that the term “ribonucleotide” or “nucleotide” can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, *e.g.*, Table 2). The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without

limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

The terms “iRNA”, “RNAi agent,” “iRNA agent,” “RNA interference agent” as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript *via* an RNA-induced silencing complex (RISC) pathway. iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The iRNA modulates, *e.g.*, inhibits, the expression of CIDEB gene in a cell, *e.g.*, a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNA that interacts with a target RNA sequence, *e.g.*, a CIDEB target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188). Thus, in one aspect the invention relates to a single stranded RNA (sssiRNA) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.*, a CIDEB gene. Accordingly, the term “siRNA” is also used herein to refer to an RNAi as described above.

In another embodiment, the RNAi agent may be a single-stranded RNAi agent that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents (ssRNAi) bind to the RISC endonuclease, Argonaute 2, which then cleaves the target mRNA. The single-stranded siRNAs are generally 15-30 nucleotides and are chemically modified. The design and testing of single-stranded RNAi agents are described in U.S. Patent No. 8,101,348 and in Lima *et al.*, (2012) *Cell* 150: 883-894, the entire contents of each of which are hereby incorporated herein by reference. Any of the antisense nucleotide sequences described herein may be used as a single-stranded siRNA as described herein or as chemically modified by the methods described in Lima *et al.*, (2012) *Cell* 150: 883-894.

In another embodiment, an “iRNA” for use in the compositions and methods of the invention is a double-stranded RNA and is referred to herein as a “double stranded RNAi agent,” “double-stranded RNA

(dsRNA) molecule,” “dsRNA agent,” or “dsRNA”. The term “dsRNA”, refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having “sense” and “antisense” orientations with respect to a target RNA, *i.e.*, a CIDEB gene. In some embodiments of the invention, a double-stranded RNA (dsRNA) triggers the degradation of a target RNA, *e.g.*, an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

In general, the majority of nucleotides of each strand of a dsRNA molecule are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide and/or a modified nucleotide. In addition, as used in this specification, an “RNAi agent” may include ribonucleotides with chemical modifications; an RNAi agent may include substantial modifications at multiple nucleotides. As used herein, the term “modified nucleotide” refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, and/or a modified nucleobase. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.*, a functional group or atom, to internucleotide linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the invention include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by “RNAi agent” for the purposes of this specification and claims.

The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 9 to 36 base pairs in length, *e.g.*, about 15-30 base pairs in length, for example, about 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, or 36 base pairs in length, such as about 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a “hairpin loop.” A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 23 or more unpaired nucleotides.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. Where the two strands are connected

covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker". The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs.

In one embodiment, an RNAi agent of the invention is a dsRNA, each strand of which comprises less than 30 nucleotides, *e.g.*, 17-27, 19-27, 17-25, 19-25, or 19-23, that interacts with a target RNA sequence, *e.g.*, a CIDEB target mRNA sequence, to direct the cleavage of the target RNA. In another embodiment, an RNAi agent of the invention is a dsRNA, each strand of which comprises 19-23 nucleotides, that interacts with a target RNA sequence, *e.g.*, a CIDEB target mRNA sequence, to direct the cleavage of the target RNA. In one embodiment, the sense strand is 21 nucleotides in length. In another embodiment, the antisense strand is 23 nucleotides in length.

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of an iRNA, *e.g.*, a dsRNA. For example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other strand, or vice versa, there is a nucleotide overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively, the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA.

In one embodiment, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end and/or the 5'-end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end and/or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In certain embodiments, the overhang on the sense strand or the antisense strand, or both, can include extended lengths longer than 10 nucleotides, *e.g.*, 10-30 nucleotides, 10-25 nucleotides, 10-20 nucleotides or 10-15 nucleotides in length. In certain embodiments, an extended overhang is on the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the sense strand of the duplex. In certain embodiments, an extended overhang is on the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the antisense strand of the duplex. In certain

embodiments, one or more of the nucleotides in the extended overhang is replaced with a nucleoside thiophosphate.

The terms “blunt” or “blunt ended” as used herein in reference to a dsRNA mean that there are no unpaired nucleotides or nucleotide analogs at a given terminal end of a dsRNA, *i.e.*, no nucleotide overhang.

5 One or both ends of a dsRNA can be blunt. Where both ends of a dsRNA are blunt, the dsRNA is said to be blunt ended. To be clear, a “blunt ended” dsRNA is a dsRNA that is blunt at both ends, *i.e.*, no nucleotide overhang at either end of the molecule. Most often such a molecule will be double-stranded over its entire length.

10 The term “antisense strand” or “guide strand” refers to the strand of an iRNA, *e.g.*, a dsRNA, which includes a region that is substantially complementary to a target sequence, *e.g.*, a CIDEB mRNA.

As used herein, the term “region of complementarity” refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, *e.g.*, a CIDEB nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most
15 tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, 3, or 2 nucleotides of the 5′- and/or 3′- terminus of the iRNA.

The term “sense strand” or “passenger strand” as used herein, refers to the strand of an iRNA that includes a region that is substantially complementary to a region of the antisense strand as that term is defined herein.

20 As used herein, the term “cleavage region” refers to a region that is located immediately adjacent to the cleavage site. The cleavage site is the site on the target at which cleavage occurs. In some embodiments, the cleavage region comprises three bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage site specifically occurs at the site bound by nucleotides
25 10 and 11 of the antisense strand, and the cleavage region comprises nucleotides 11, 12 and 13.

As used herein, and unless otherwise indicated, the term “complementary,” when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as
30 will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing (see, *e.g.*, “Molecular Cloning: A Laboratory Manual, Sambrook, *et al.* (1989) Cold Spring Harbor Laboratory Press). Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled person will be able to determine the set of

conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

Complementary sequences within an iRNA, *e.g.*, within a dsRNA as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of one or both nucleotide sequences. Such sequences can be referred to as “fully complementary” with respect to each other herein. However, where a first sequence is referred to as “substantially complementary” with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3 or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, *e.g.*, inhibition of gene expression via a RISC pathway. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as “fully complementary” for the purposes described herein.

“Complementary” sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogsteen base pairing.

The terms “complementary,” “fully complementary” and “substantially complementary” herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of an iRNA agent and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is “substantially complementary to at least part of” a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding CIDEB). For example, a polynucleotide is complementary to at least a part of a CIDEB mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding CIDEB.

Accordingly, in some embodiments, the antisense strand polynucleotides disclosed herein are fully complementary to the target CIDEB sequence. In other embodiments, the antisense strand polynucleotides disclosed herein are substantially complementary to the target CIDEB sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NO:1, or a fragment of SEQ ID NO:1, such as about 85%, about 86%,

about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In one embodiment, an RNAi agent of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target CIDEB sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NOs: 2, or a fragment of any one of SEQ ID NOs: 2, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In some embodiments, an iRNA of the invention includes an antisense strand that is substantially complementary to the target CIDEB sequence and comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of the sense strands in Tables 3-6, or a fragment of any one of the sense strands in Tables 3-6, such as about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% complementary, or 100% complementary.

The term “inhibiting,” as used herein, is used interchangeably with “reducing,” “silencing,” “downregulating,” “suppressing” and other similar terms, and includes any level of inhibition.

The phrase “inhibiting expression of a CIDEB gene,” as used herein, includes inhibition of expression of any CIDEB gene (such as, *e.g.*, a mouse CIDEB gene, a rat CIDEB gene, a monkey CIDEB gene, or a human CIDEB gene) as well as variants or mutants of a CIDEB gene that encode a CIDEB protein.

“Inhibiting expression of a CIDEB gene” includes any level of inhibition of a CIDEB gene, *e.g.*, at least partial suppression of the expression of a CIDEB gene, such as an inhibition by at least about 20%. In certain embodiments, inhibition is by at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

The expression of a CIDEB gene may be assessed based on the level of any variable associated with CIDEB gene expression, *e.g.*, CIDEB mRNA level or CIDEB protein level. The expression of a CIDEB gene may also be assessed indirectly based on, for example, a decrease in CIDEB protein activity, *e.g.*, a decrease in the interaction of CIDEB with ApoB and/or a decrease in lipid maturation in a tissue sample, such as a liver sample. Inhibition may be assessed by a decrease in an absolute or relative level of one or more of these variables compared with a control level. The control level may be any type of control level that is utilized in the art, *e.g.*, a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

In one embodiment, at least partial suppression of the expression of a CIDEB gene, is assessed by a reduction of the amount of CIDEB mRNA which can be isolated from, or detected, in a first cell or group of cells in which a CIDEB gene is transcribed and which has or have been treated such that the expression of a CIDEB gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells).

The degree of inhibition may be expressed in terms of:

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

The phrase “contacting a cell with an RNAi agent,” such as a dsRNA, as used herein, includes contacting a cell by any possible means. Contacting a cell with an RNAi agent includes contacting a cell *in vitro* with the iRNA or contacting a cell *in vivo* with the iRNA. The contacting may be done directly or indirectly. Thus, for example, the RNAi agent may be put into physical contact with the cell by the individual performing the method, or alternatively, the RNAi agent may be put into a situation that will permit or cause it to subsequently come into contact with the cell.

Contacting a cell *in vitro* may be done, for example, by incubating the cell with the RNAi agent. Contacting a cell *in vivo* may be done, for example, by injecting the RNAi agent into or near the tissue where the cell is located, or by injecting the RNAi agent into another area, *e.g.*, the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the RNAi agent may contain and/or be coupled to a ligand, *e.g.*, GalNAc3, that directs the RNAi agent to a site of interest, *e.g.*, the liver. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. For example, a cell may also be contacted *in vitro* with an RNAi agent and subsequently transplanted into a subject.

In one embodiment, contacting a cell with an iRNA includes “introducing” or “delivering the iRNA into the cell” by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an iRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices.

Introducing an iRNA into a cell may be *in vitro* and/or *in vivo*. For example, for *in vivo* introduction, iRNA can be injected into a tissue site or administered systemically. *In vivo* delivery can also be done by a beta-glucan delivery system, such as those described in U.S. Patent Nos. 5,032,401 and 5,607,677, and U.S. Publication No. 2005/0281781, the entire contents of which are hereby incorporated herein by reference. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection.

Further approaches are described herein below and/or are known in the art.

The term “lipid nanoparticle” or “LNP” is a vesicle comprising a lipid layer encapsulating a pharmaceutically active molecule, such as a nucleic acid molecule, *e.g.*, an iRNA or a plasmid from which an iRNA is transcribed. LNPs are described in, for example, U.S. Patent Nos. 6,858,225, 6,815,432, 8,158,601, and 8,058,069, the entire contents of which are hereby incorporated herein by reference.

As used herein, a “subject” is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), a non-primate (such as a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, a horse, and a whale), or a bird (*e.g.*, a duck or a goose).

In an embodiment, the subject is a human, such as a human being treated or assessed for a disease, disorder or condition that would benefit from reduction in CIDEB expression; a human at risk for a disease, disorder or condition that would benefit from reduction in CIDEB expression; a human having a disease, disorder or condition that would benefit from reduction in CIDEB expression; and/or human being treated for a disease, disorder or condition that would benefit from reduction in CIDEB expression as described herein.

As used herein, the terms “treating” or “treatment” refer to a beneficial or desired result including, but not limited to, alleviation or amelioration of one or more symptoms associated with CIDEB gene expression and/or CIDEB protein production, *e.g.*, a CIDEB-associated disease, such as a chronic inflammatory disease of the liver and other tissues. In one embodiment, the chronic inflammatory disease is chronic inflammatory liver disease, *e.g.*, inflammation of the liver, liver fibrosis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), cirrhosis of the liver, alcoholic steatohepatitis (ASH), alcoholic liver diseases (ALD), HCV-associated cirrhosis, drug induced liver injury, hepatocellular necrosis, hepatocellular carcinoma, insulin insensitivity and/or diabetes. “Treatment” can also mean prolonging survival as compared to expected survival in the absence of treatment.

The term “lower” in the context of a CIDEB-associated disease refers to a statistically significant decrease in such level. The decrease can be, for example, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or more. In certain embodiments, a decrease is at least 20%. “Lower” in the context of the level of CIDEB in a subject is preferably down to a level accepted as within the range of normal for an individual without such disorder.

As used herein, “prevention” or “preventing,” when used in reference to a disease, disorder or condition thereof, that would benefit from a reduction in expression of a CIDEB gene, refers to a reduction in the likelihood that a subject will develop a symptom associated with such disease, disorder, or condition, *e.g.*, a symptom of CIDEB gene expression, such as inflammation of the liver, liver fibrosis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), cirrhosis of the liver, alcoholic steatohepatitis (ASH), alcoholic liver diseases (ALD), HCV-associated cirrhosis, drug induced liver injury, hepatocellular necrosis, hepatocellular carcinoma, insulin insensitivity and/or diabetes. The failure to develop a disease, disorder or condition, or the reduction in the development of a symptom associated with such a disease, disorder or condition (*e.g.*, by at least about 10% on a clinically accepted scale for that disease or disorder), or the exhibition of delayed symptoms (*e.g.*, reduction in inflammation, or reduction in lipid

accumulation in the liver and/or lipid droplet expansion in the liver) delayed (e.g., by days, weeks, months or years) is considered effective prevention.

As used herein, the term "CIDEB-associated disease," is a disease or disorder that is caused by, or associated with, CIDEB gene expression or CIDEB protein production. The term "CIDEB-associated disease" includes a disease, disorder or condition that would benefit from a decrease in CIDEB gene expression or protein activity.

In one embodiment, an "CIDEB-associated disease" is a chronic inflammatory disease. A "chronic inflammatory disease" is any disease, disorder, or condition associated with chronic inflammation. Non-limiting examples of a chronic inflammatory disease include, for example, inflammation of the liver and other tissues. Non-limiting examples of chronic inflammatory liver disease include, for example, fibrosis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), cirrhosis of the liver, alcoholic steatohepatitis (ASH), alcoholic liver diseases (ALD), HCV-associated cirrhosis, drug induced liver injury, hepatocellular necrosis, hepatocellular carcinoma, insulin insensitivity and/or diabetes.

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a CIDEB-associated disease, disorder, or condition, is sufficient to effective treatment of the disease (e.g., by diminishing, ameliorating or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an iRNA that, when administered to a subject having a CIDEB-associated disease, disorder, or condition, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the iRNA, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "prophylactically effective amount" also includes an amount of an RNAi agent that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. iRNA employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human subjects and animal subjects without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In certain embodiments, samples may be derived from the liver (*e.g.*, whole liver or certain segments of liver or certain types of cells in the liver, such as, *e.g.*, hepatocytes). In some embodiments, a "sample derived from a subject" refers to blood or plasma drawn from the subject.

II. iRNAs of the Invention

Described herein are iRNAs which inhibit the expression of a target gene. In one embodiment, the iRNAs inhibit the expression of a CIDEB gene. In one embodiment, the iRNA agent includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a CIDEB gene in a cell, such as a liver cell, such as a liver cell within a subject, *e.g.*, a mammal, such as a human having a chronic inflammatory disease, disorder, or condition.

The dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of a CIDEB gene. The region of complementarity is

about 30 nucleotides or less in length (*e.g.*, about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 nucleotides or less in length). Upon contact with a cell expressing the target gene, the iRNA inhibits the expression of the target gene (*e.g.*, a human, a primate, a non-primate, or a rodent target gene) by at least about 10% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, Western Blotting or flowcytometric techniques.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of a CIDEB gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

Generally, the duplex structure is between 15 and 30 base pairs in length, *e.g.*, between, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

Similarly, the region of complementarity to the target sequence is between 15 and 30 nucleotides in length, *e.g.*, between 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

In some embodiments, the sense and antisense strands of the dsRNA are each independently about 15 to about 30 nucleotides in length, or about 25 to about 30 nucleotides in length, *e.g.*, each strand is independently between 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. In some embodiments, the dsRNA is between about 15 and about 23 nucleotides in length, or between about 25

and about 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well known in the art that dsRNAs longer than about 21-23 nucleotides can serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a
5 “part” of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.*, a duplex region of about 9 to 36 base pairs, *e.g.*, about 10-36, 11-36, 12-36, 13-36, 14-36, 15-36, 9-35, 10-35, 11-35, 12-35, 13-35, 14-35, 15-35, 9-34, 10-34, 11-34, 12-34, 13-34, 14-34, 15-34, 9-33, 10-33, 10 11-33, 12-33, 13-33, 14-33, 15-33, 9-32, 10-32, 11-32, 12-32, 13-32, 14-32, 15-32, 9-31, 10-31, 11-31, 12-31, 13-32, 14-31, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs. Thus, in one
15 embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a MicroRNA (miRNA) is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target CIDEA expression is not
20 generated in the target cell by cleavage of a larger dsRNA.

A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs *e.g.*, 1, 2, 3, or 4 nucleotides. dsRNAs having at least one nucleotide overhang can have unexpectedly superior inhibitory properties relative to their blunt-ended counterparts. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be
25 on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA.

A dsRNA can be synthesized by standard methods known in the art as further discussed below, *e.g.*, by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

30 iRNA compounds of the invention may be prepared using a two-step procedure. First, the individual strands of the double-stranded RNA molecule are prepared separately. Then, the component strands are annealed. The individual strands of the siRNA compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide strands comprising unnatural or modified nucleotides can be easily prepared. Single-stranded oligonucleotides of the invention
35 can be prepared using solution-phase or solid-phase organic synthesis or both.

In one aspect, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an antisense sequence. The sense strand sequence is selected from the group of sequences provided in Tables 3-6, and the corresponding nucleotide sequence of the antisense strand of the sense strand is selected from the group of sequences of Tables 3-6. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a CIDEB gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand (passenger strand) in Tables 3-6, and the second oligonucleotide is described as the corresponding antisense strand (guide strand) of the sense strand in Tables 3-6. In one embodiment, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

It will be understood that, although the sequences in Tables 3-6 are described as modified, unmodified, unconjugated, and/or conjugated sequences, the RNA of the invention *e.g.*, a dsRNA of the invention, may comprise any one of the sequences set forth Tables 3-6 that is un-modified, unconjugated, and/or modified and/or conjugated differently than described therein.

The skilled person is well aware that dsRNAs having a duplex structure of between about 20 and 23 base pairs, *e.g.*, 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, (2001) *EMBO J.*, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided herein, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences provided herein, and differing in their ability to inhibit the expression of a CIDEB gene by not more than about 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the RNAs described in Tables 3-6 identify a site(s) in a CIDEB transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within this site(s). As used herein, an iRNA is said to target within a particular site of an RNA transcript if the iRNA promotes cleavage of the transcript anywhere within that particular site. Such an iRNA will generally include at least about 15 contiguous nucleotides from one of the sequences provided herein coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in the gene.

While a target sequence is generally about 15-30 nucleotides in length, there is wide variation in the suitability of particular sequences in this range for directing cleavage of any given target RNA. Various

software packages and the guidelines set out herein provide guidance for the identification of optimal target sequences for any given gene target, but an empirical approach can also be taken in which a “window” or “mask” of a given size (as a non-limiting example, 21 nucleotides) is literally or figuratively (including, *e.g.*, in silico) placed on the target RNA sequence to identify sequences in the size range that can serve as target sequences. By moving the sequence “window” progressively one nucleotide upstream or downstream of an initial target sequence location, the next potential target sequence can be identified, until the complete set of possible sequences is identified for any given target size selected. This process, coupled with systematic synthesis and testing of the identified sequences (using assays as described herein or as known in the art) to identify those sequences that perform optimally can identify those RNA sequences that, when targeted with an iRNA agent, mediate the best inhibition of target gene expression. Thus, while the sequences identified herein represent effective target sequences, it is contemplated that further optimization of inhibition efficiency can be achieved by progressively “walking the window” one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.

Further, it is contemplated that for any sequence identified herein, further optimization could be achieved by systematically either adding or removing nucleotides to generate longer or shorter sequences and testing those sequences generated by walking a window of the longer or shorter size up or down the target RNA from that point. Again, coupling this approach to generating new candidate targets with testing for effectiveness of iRNAs based on those target sequences in an inhibition assay as known in the art and/or as described herein can lead to further improvements in the efficiency of inhibition. Further still, such optimized sequences can be adjusted by, *e.g.*, the introduction of modified nucleotides as described herein or as known in the art, addition or changes in overhang, or other modifications as known in the art and/or discussed herein to further optimize the molecule (*e.g.*, increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, targeting to a particular location or cell type, increasing interaction with silencing pathway enzymes, increasing release from endosomes) as an expression inhibitor.

An iRNA agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, an iRNA as described herein contains no more than 3 mismatches. If the antisense strand of the iRNA contains mismatches to a target sequence, it is preferable that the area of mismatch is not located in the center of the region of complementarity. If the antisense strand of the iRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, for a 23 nucleotide iRNA agent the strand which is complementary to a region of a CIDEB gene, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an iRNA containing a mismatch to a target sequence is effective in inhibiting the expression of a CIDEB gene. Consideration of the efficacy of iRNAs with mismatches in inhibiting expression of a CIDEB gene is

important, especially if the particular region of complementarity in a CIDEB gene is known to have polymorphic sequence variation within the population.

An RNA target may have regions, or spans of the target RNA's nucleotide sequence, which are relatively more susceptible or amenable than other regions of the RNA target to mediating cleavage of the RNA target via RNA interference induced by the binding of an RNAi agent to that region. The increased susceptibility to RNA interference within such "hotspot regions" (or simply "hotspots") means that iRNA agents targeting the region will likely have higher efficacy in inducing iRNA interference than iRNA agents which target other regions of the target RNA. For example, without being bound by theory, the accessibility of a target region of a target RNA may influence the efficacy of iRNA agents which target that region, with some hotspot regions having increased accessibility. Secondary structures, for instance, that form in the RNA target (e.g., within or proximate to hotspot regions) may affect the ability of the iRNA agent to bind the target region and induce RNA interference.

According to certain aspects of the invention, an iRNA agent may be designed to target a hotspot region of any of the target RNAs described herein, including any identified portions of a target RNA (e.g., a particular exon). As used herein, a hotspot region may refer to an approximately 19-200, 19-150, 19-100, 19-75, 19-50, 21-200, 21-150, 21-100, 21-75, 21-50, 50-200, 50-150, 50-100, 50-75, 75-200, 75-150, 75-100, 100-200, or 100-150 nucleotide region of a target RNA sequence for which targeting using RNAi agents provides an observably higher probability of efficacious silencing relative to targeting other regions of the same target RNA. According to certain aspects of the invention, a hotspot region may comprise a limited region of the target RNA, and in some cases, a substantially limited region of the target, including for example, less than half of the length of the target RNA, such as about 5%, 10%, 15%, 20%, 25%, or 30% of the length of the target RNA. Conversely, the other regions against which a hotspot is compared may cumulatively comprise at least a majority of the length of the target RNA. For example, the other regions may cumulatively comprise at least about 60%, or at least about 70%, or at least about 80%, or at least about 90%, or at least about 95% of the length of the target RNA.

Compared regions of the target RNA may be empirically evaluated for identification of hotspots using efficacy data obtained from *in vitro* or *in vivo* screening assays. For example, RNAi agents targeting various regions that span a target RNA may be compared for frequency of efficacious iRNA agents (e.g., the amount by which target gene expression is inhibited, such as measured by mRNA expression or protein expression) that bind each region. In general, a hotspot can be recognized by observing clustering of multiple efficacious RNAi agents that bind to a limited region of the RNA target. A hotspot may be sufficiently characterized as such by observing efficacy of iRNA agents which cumulatively span at least about 60% of the target region identified as a hotspot, such as about 70%, about 80%, about 90%, or about 95% or more of the length of the region, including both ends of the region (i.e. at least about 60%, 70%, 80%, 90%, or 95% or more of the nucleotides within the region, including the nucleotides at each end of the region, were targeted by an iRNA

agent). According to some aspects of the invention, an iRNA agent which demonstrates at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% inhibition over the region (e.g., no more than about 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% mRNA remaining) may be identified as efficacious.

5 Amenability to targeting of RNA regions may also be assessed using quantitative comparison of inhibition measurements across different regions of a defined size (e.g., 25, 30, 40, 50, 60, 70, 80, 90, or 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 nts). For example, an average level of inhibition may be determined for each region and the averages of each region may be compared. The average level of inhibition within a hotspot region may be substantially higher than the average of averages for all evaluated regions.

10 According to some aspects, the average level of inhibition in a hotspot region may be at least about 10%, 20%, 30%, 40%, or 50% higher than the average of averages. According to some aspects, the average level of inhibition in a hotspot region may be at least about 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2.0 standard deviations above the average of averages. The average level of inhibition may be higher by a statistically significant (e.g., $p < 0.05$) amount. According to some aspects, each inhibition measurement within a hotspot

15 region may be above a threshold amount (e.g., at or below a threshold amount of mRNA remaining). According to some aspects, each inhibition measurement within the region may be substantially higher than an average of all inhibition measurements across all the measured regions. For example, each inhibition measurement in a hotspot region may be at least about 10%, 20%, 30%, 40%, or 50% higher than the average of all inhibition measurements. According to some aspects, each inhibition measurement may be at least

20 about 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2.0 standard deviations above the average of all inhibition measurements. Each inhibition measurement may be higher by a statistically significant (e.g., $p < 0.05$) amount than the average of all inhibition measurements. A standard for evaluating a hotspot may comprise various combinations of the above standards where compatible (e.g., an average level of inhibition of at least about a first amount and having no inhibition measurements below a threshold level of a second amount,

25 lesser than the first amount).

It is therefore expressly contemplated that any iRNA agent, including the specific exemplary iRNA agents described herein, which targets a hotspot region of a target RNA, may be preferably selected for inducing RNA interference of the target mRNA as targeting such a hotspot region is likely to exhibit a robust inhibitory response relative to targeting a region which is not a hotspot region. RNAi agents targeting target

30 sequences that substantially overlap (e.g., by at least about 70%, 75%, 80%, 85%, 90%, 95% of the target sequence length) or, preferably, that reside fully within the hotspot region may be considered to target the hotspot region. Hotspot regions of the RNA target(s) of the instant invention may include any region for which the data disclosed herein demonstrates higher frequency of targeting by efficacious RNAi agents, including by any of the standards described elsewhere herein, whether or not the range(s) of such hotspot

35 region(s) are explicitly specified.

In various embodiments, a dsRNA agent of the present invention targets a hotspot region of an mRNA encoding CIDEB.

III. Modified iRNAs of the Invention

5 In one embodiment, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is un-modified, and does not comprise, *e.g.*, chemical modifications and/or conjugations known in the art and described herein. In another embodiment, the RNA of an iRNA of the invention, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the invention, substantially all of the nucleotides of an iRNA of the invention are modified. In other embodiments of the invention, all of the
10 nucleotides of an iRNA of the invention are modified. iRNAs of the invention in which “substantially all of the nucleotides are modified” are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

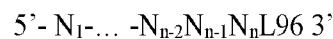
In some aspects of the invention, substantially all of the nucleotides of an iRNA of the invention are modified and the iRNA agents comprise no more than 10 nucleotides comprising 2'-fluoro modifications (*e.g.*,
15 no more than 9 2'-fluoro modifications, no more than 8 2'-fluoro modifications, no more than 7 2'-fluoro modifications, no more than 6 2'-fluoro modifications, no more than 5 2'-fluoro modifications, no more than 4 2'-fluoro modifications, no more than 5 2'-fluoro modifications, no more than 4 2'-fluoro modifications, no more than 3 2'-fluoro modifications, or no more than 2 2'-fluoro modifications). For example, in some embodiments, the sense strand comprises no more than 4 nucleotides comprising 2'-fluoro modifications (*e.g.*,
20 no more than 3 2'-fluoro modifications, or no more than 2 2'-fluoro modifications). In other embodiments, the antisense strand comprises no more than 6 nucleotides comprising 2'-fluoro modifications (*e.g.*, no more than 5 2'-fluoro modifications, no more than 4 2'-fluoro modifications, no more than 4 2'-fluoro modifications, or no more than 2 2'-fluoro modifications).

In other aspects of the invention, all of the nucleotides of an iRNA of the invention are modified and
25 the iRNA agents comprise no more than 10 nucleotides comprising 2'-fluoro modifications (*e.g.*, no more than 9 2'-fluoro modifications, no more than 8 2'-fluoro modifications, no more than 7 2'-fluoro modifications, no more than 6 2'-fluoro modifications, no more than 5 2'-fluoro modifications, no more than 4 2'-fluoro modifications, no more than 5 2'-fluoro modifications, no more than 4 2'-fluoro modifications, no more than 3 2'-fluoro modifications, or no more than 2 2'-fluoro modifications).

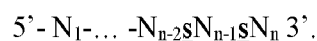
30 In one embodiment, the double stranded RNAi agent of the invention further comprises a 5'-phosphate or a 5'-phosphate mimic at the 5' nucleotide of the antisense strand. In another embodiment, the double stranded RNAi agent further comprises a 5'-phosphate mimic at the 5' nucleotide of the antisense strand. In a specific embodiment, the 5'-phosphate mimic is a 5'-vinyl phosphonate (5'-VP). In one

embodiment, the phosphate mimic is a 5'-cyclopropyl phosphonate. In some embodiments, the 5'-end of the antisense strand of the double-stranded iRNA agent does not contain a 5'-vinyl phosphonate (VP).

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-
 5 modified nucleotide, a glycol modified nucleotide (GNA), e.g., Ggn, Cgn, Tgn, or Agn, a nucleotide with a 2' phosphate, e.g., G2p, C2p, A2p or U2p, and, a vinyl-phosphonate nucleotide; and combinations thereof. In other embodiments, each of the duplexes of Tables 4 and 6 may be particularly modified to provide another double-stranded iRNA agent of the present disclosure. In one example, the 3'-terminus of each
 10 sense duplex may be modified by removing the 3'-terminal L96 ligand and exchanging the two phosphodiester internucleotide linkages between the three 3'-terminal nucleotides with phosphorothioate internucleotide linkages. That is, the three 3'-terminal nucleotides (N) of a sense sequence of the formula:



may be replaced with



15 That is, for example, AD-1685156, the sense sequence:

csusgcagAfaGfGfUfugacugcguuL96 (SEQ ID NO: 277)

may be replaced with

csusgcagAfaGfGfUfugacugcgsusu (SEQ ID NO: 2922)

20 while the antisense sequence remains unchanged to provide another double-stranded iRNA agent of the present disclosure.

The nucleic acids featured in the invention can be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Modifications include, for example, end modifications, e.g., 5'-end modifications
 25 (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*); base modifications, e.g., replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (e.g., at the 2'-position or 4'-position) or replacement of the sugar; and/or backbone modifications, including modification or replacement of the phosphodiester linkages.
 30 Specific examples of iRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having

modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified iRNA will have a phosphorus atom in its internucleoside backbone.

5 Modified RNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and
10 those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. In some embodiments of the invention, the dsRNA agents of the invention are in a free acid form. In other embodiments of the invention, the dsRNA agents of the invention are in a salt form. In one embodiment, the dsRNA agents of the invention are in a sodium salt form. In certain embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present
15 in the agent as counterions for substantially all of the phosphodiester and/or phosphorothioate groups present in the agent. Agents in which substantially all of the phosphodiester and/or phosphorothioate linkages have a sodium counterion include not more than 5, 4, 3, 2, or 1 phosphodiester and/or phosphorothioate linkages without a sodium counterion. In some embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for all of the phosphodiester and/or phosphorothioate groups present in
20 the agent.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6,239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and US Pat RE39464, the entire contents of each of which are hereby incorporated herein by reference.

30 Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones;

methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Patent Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

In other embodiments, suitable RNA mimetics are contemplated for use in iRNAs, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an RNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the iRNAs of the invention are described in, for example, in Nielsen *et al.*, *Science*, 1991, 254, 1497-1500.

Some embodiments featured in the invention include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH₂--NH--CH₂--, --CH₂--N(CH₃)--O--CH₂-- [known as a methylene (methylimino) or MMI backbone], --CH₂--O--N(CH₃)--CH₂--, --CH₂--N(CH₃)--N(CH₃)--CH₂-- and --N(CH₃)--CH₂--CH₂-- [wherein the native phosphodiester backbone is represented as --O--P--O--CH₂--] of the above-referenced U.S. Patent No. 5,489,677, and the amide backbones of the above-referenced U.S. Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506.

Modified RNAs can also contain one or more substituted sugar moieties. The iRNAs, *e.g.*, dsRNAs, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Exemplary suitable modifications include O[(CH₂)_nO]_mCH₃, O(CH₂)_nOCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an iRNA, or

a group for improving the pharmacodynamic properties of an iRNA, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O--CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminoethoxyethoxy, *i.e.*, a

5 O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH₂--O--CH₂--N(CH₂)₂. Further exemplary modifications include: 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

10 Other modifications include 2'-methoxy (2'-OCH₃), 2'-aminopropoxy (2'-OCH₂CH₂CH₂NH₂) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the RNA of an iRNA, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. iRNAs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures

15 include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application. The entire contents of each of the foregoing are hereby incorporated herein by reference.

20 An iRNA of the invention can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of

25 adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine

30 and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L. ed. John Wiley & Sons, 1990, those disclosed by Englisch *et al.*, (1991) *Angewandte Chemie, International Edition*, 30:613, and those disclosed by Sanghvi, Y

35 S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC

Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2 °C (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., dsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Patent Nos. 3,687,808, 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

An iRNA of the invention can also be modified to include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193).

An iRNA of the invention can also be modified to include one or more bicyclic sugar moities. A "bicyclic sugar" is a furanosyl ring modified by the bridging of two atoms. A "bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring. Thus, in some embodiments an agent of the invention may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH₂-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the invention include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the invention

include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to 4'-(CH₂)—O-2' (LNA); 4'-(CH₂)—S-2'; 4'-(CH₂)₂—O-2' (ENA); 4'-CH(CH₃)—O-2' (also referred to as "constrained ethyl" or "cEt") and 4'-CH(CH₂OCH₃)—O-2' (and analogs thereof; see, *e.g.*, U.S. Pat. No. 7,399,845); 4'-C(CH₃)(CH₃)—O-2' (and analogs thereof; see *e.g.*, US Patent No. 8,278,283); 4'-CH₂—N(OCH₃)-2' (and analogs thereof; see *e.g.*, US Patent No. 8,278,425); 4'-CH₂—O—N(CH₃)-2' (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4'-CH₂—N(R)—O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting group (see, *e.g.*, U.S. Pat. No. 7,427,672); 4'-CH₂—C(H)(CH₃)-2' (see, *e.g.*, Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂—C(=CH₂)-2' (and analogs thereof; see, *e.g.*, US Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

Additional representative U.S. Patents and US Patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133; 7,084,125; 7,399,845; 7,427,672; 7,569,686; 7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283; US 2008/0039618; and US 2009/0012281, the entire contents of each of which are hereby incorporated herein by reference.

Any of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α -L-ribofuranose and β -D-ribofuranose (see WO 99/14226).

An iRNA of the invention can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH₃)-O-2' bridge. In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

An iRNA of the invention may also include one or more "conformationally restricted nucleotides" ("CRN"). CRN are nucleotide analogs with a linker connecting the C2' and C4' carbons of ribose or the C3 and -C5' carbons of ribose. CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, US Patent Publication No. 2013/0190383; and PCT publication WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

In some embodiments, an iRNA of the invention comprises one or more monomers that are UNA (unlocked nucleic acid) nucleotides. UNA is unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'-C4' have been removed (i.e. the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (i.e. the covalent carbon-carbon bond

between the C2' and C3' carbons) of the sugar has been removed (see *Nuc. Acids Symp. Series*, 52, 133-134 (2008) and Fluiter et al., *Mol. Biosyst.*, 2009, 10, 1039 hereby incorporated by reference).

Representative U.S. publications that teach the preparation of UNA include, but are not limited to, US Patent No. 8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

An RNAi agent of the disclosure may also include one or more "cyclohexene nucleic acids" or ("CeNA"). CeNA are nucleotide analogs with a replacement of the furanose moiety of DNA by a cyclohexene ring. Incorporation of cyclohexenyl nucleosides in a DNA chain increases the stability of a DNA/RNA hybrid. CeNA is stable against degradation in serum and a CeNA/RNA hybrid is able to activate E. Coli RNase H, resulting in cleavage of the RNA strand. (see Wang et al., *Am. Chem. Soc.* 2000, 122, 36, 8595-8602, hereby incorporated by reference).

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-O-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3'-phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

Other modifications of an iRNA of the invention include a 5' phosphate or 5' phosphate mimic, e.g., a 5'-terminal phosphate or phosphate mimic on the antisense strand of an RNAi agent. Suitable phosphate mimics are disclosed in, for example US Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

In certain specific embodiments, an RNAi agent of the present invention is an agent that inhibits the expression of a CIDEB gene which is selected from the group of agents listed in Tables 3-6. Any of these agents may further comprise a ligand.

A. Modified iRNAs Comprising Motifs of the Invention

In certain aspects of the invention, the double stranded RNAi agents of the invention include agents with chemical modifications as disclosed, for example, in WO 2013/075035, filed on November 16, 2012, the entire contents of which are incorporated herein by reference.

Accordingly, the invention provides double stranded RNAi agents capable of inhibiting the expression of a target gene (i.e., a CIDEB gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may range from 12-30 nucleotides in length. For example, each strand may be between 14-30 nucleotides in length, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 17-23 nucleotides in length, 17-21 nucleotides in length, 17-19 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in

length, or 21-23 nucleotides in length. In one embodiment, the sense strand is 21 nucleotides in length. In one embodiment, the antisense strand is 23 nucleotides in length.

The sense strand and antisense strand typically form a duplex double stranded RNA ("dsRNA"), also referred to herein as an "RNAi agent." The duplex region of an RNAi agent may be 12-30 nucleotide pairs in length. For example, the duplex region can be between 14-30 nucleotide pairs in length, 17-30 nucleotide pairs in length, 27-30 nucleotide pairs in length, 17 - 23 nucleotide pairs in length, 17-21 nucleotide pairs in length, 17-19 nucleotide pairs in length, 19-25 nucleotide pairs in length, 19-23 nucleotide pairs in length, 19-21 nucleotide pairs in length, 21-25 nucleotide pairs in length, or 21-23 nucleotide pairs in length. In another example, the duplex region is selected from 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27 nucleotides in length.

In one embodiment, the RNAi agent may contain one or more overhang regions and/or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The overhang can be 1-6 nucleotides in length, for instance 2-6 nucleotides in length, 1-5 nucleotides in length, 2-5 nucleotides in length, 1-4 nucleotides in length, 2-4 nucleotides in length, 1-3 nucleotides in length, 2-3 nucleotides in length, or 1-2 nucleotides in length. The overhangs can be the result of one strand being longer than the other, or the result of two strands of the same length being staggered. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence. The first and second strands can also be joined, *e.g.*, by additional bases to form a hairpin, or by other non-base linkers.

In one embodiment, the nucleotides in the overhang region of the RNAi agent can each independently be a modified or unmodified nucleotide including, but not limited to 2'-sugar modified, such as, 2'-F, 2'-Omethyl, thymidine (T), 2'-O-methoxyethyl-5-methyluridine (Teo), 2'-O-methoxyethyladenosine (Aeo), 2'-O-methoxyethyl-5-methylcytidine (m5Ceo), and any combinations thereof. For example, TT can be an overhang sequence for either end on either strand. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence.

The 5'- or 3'- overhangs at the sense strand, antisense strand or both strands of the RNAi agent may be phosphorylated. In some embodiments, the overhang region(s) contains two nucleotides having a phosphorothioate between the two nucleotides, where the two nucleotides can be the same or different. In one embodiment, the overhang is present at the 3'-end of the sense strand, antisense strand, or both strands. In one embodiment, this 3'-overhang is present in the antisense strand. In one embodiment, this 3'-overhang is present in the sense strand.

The RNAi agent may contain only a single overhang, which can strengthen the interference activity of the RNAi, without affecting its overall stability. For example, the single-stranded overhang may be located at the 3'-terminal end of the sense strand or, alternatively, at the 3'-terminal end of the antisense strand. The RNAi may also have a blunt end, located at the 5'-end of the antisense strand (or the 3'-end of the sense strand) or *vice versa*. Generally, the antisense strand of the RNAi has a nucleotide overhang at the 3'-end, and

the 5'-end is blunt. While not wishing to be bound by theory, the asymmetric blunt end at the 5'-end of the antisense strand and 3'-end overhang of the antisense strand favor the guide strand loading into RISC process.

In one embodiment, the RNAi agent is a double ended bluntmer of 19 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 7, 8, 9 from the 5' end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5' end.

In another embodiment, the RNAi agent is a double ended bluntmer of 20 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 8, 9, 10 from the 5' end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5' end.

In yet another embodiment, the RNAi agent is a double ended bluntmer of 21 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5' end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5' end.

In one embodiment, the RNAi agent comprises a 21 nucleotide sense strand and a 23 nucleotide antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5' end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5' end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nucleotide overhang.

Preferably, the 2 nucleotide overhang is at the 3'-end of the antisense strand.

When the 2 nucleotide overhang is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In one embodiment, every nucleotide in the sense strand and the antisense strand of the RNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In one embodiment each residue is independently modified with a 2'-O-methyl or 3'-fluoro, *e.g.*, in an alternating motif. Optionally, the RNAi agent further comprises a ligand (preferably GalNAc₃).

In one embodiment, the RNAi agent comprises a sense and an antisense strand, wherein the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1) positions 1 to 23 of the first strand comprise at least 8 ribonucleotides; the antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, comprises at least 8 ribonucleotides in the positions paired with positions 1-23 of sense strand to form a duplex; wherein at least the 3' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides

are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when the double stranded nucleic acid is introduced into a mammalian cell; and wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

In one embodiment, the RNAi agent comprises sense and antisense strands, wherein the RNAi agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11, 12, 13 from the 5' end; wherein the 3' end of the first strand and the 5' end of the second strand form a blunt end and the second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region which is at least 25 nucleotides in length, and the second strand is sufficiently complementary to a target mRNA along at least 19 nucleotide of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein dicer cleavage of the RNAi agent preferentially results in an siRNA comprising the 3' end of the second strand, thereby reducing expression of the target gene in the mammal. Optionally, the RNAi agent further comprises a ligand.

In one embodiment, the sense strand of the RNAi agent contains at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at the cleavage site in the sense strand.

In one embodiment, the antisense strand of the RNAi agent can also contain at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at or near the cleavage site in the antisense strand.

For an RNAi agent having a duplex region of 17-23 nucleotide in length, the cleavage site of the antisense strand is typically around the 10, 11 and 12 positions from the 5'-end. Thus the motifs of three identical modifications may occur at the 9, 10, 11 positions; 10, 11, 12 positions; 11, 12, 13 positions; 12, 13, 14 positions; or 13, 14, 15 positions of the antisense strand, the count starting from the 1st nucleotide from the 5'-end of the antisense strand, or, the count starting from the 1st paired nucleotide within the duplex region from the 5'-end of the antisense strand. The cleavage site in the antisense strand may also change according to the length of the duplex region of the RNAi from the 5'-end.

The sense strand of the RNAi agent may contain at least one motif of three identical modifications on three consecutive nucleotides at the cleavage site of the strand; and the antisense strand may have at least one motif of three identical modifications on three consecutive nucleotides at or near the cleavage site of the strand. When the sense strand and the antisense strand form a dsRNA duplex, the sense strand and the antisense strand can be so aligned that one motif of the three nucleotides on the sense strand and one motif of the three nucleotides on the antisense strand have at least one nucleotide overlap, *i.e.*, at least one of the three nucleotides of the motif in the sense strand forms a base pair with at least one of the three nucleotides of the motif in the antisense strand. Alternatively, at least two nucleotides may overlap, or all three nucleotides may overlap.

In one embodiment, the sense strand of the RNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides. The first motif may occur at or near the cleavage site of the strand and the other motifs may be a wing modification. The term “wing modification” herein refers to a motif occurring at another portion of the strand that is separated from the motif at or near the cleavage site of the same strand. The wing modification is either adjacent to the first motif or is separated by at least one or more nucleotides. When the motifs are immediately adjacent to each other then the chemistry of the motifs are distinct from each other and when the motifs are separated by one or more nucleotide then the chemistries can be the same or different. Two or more wing modifications may be present. For instance, when two wing modifications are present, each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

Like the sense strand, the antisense strand of the RNAi agent may contain more than one motifs of three identical modifications on three consecutive nucleotides, with at least one of the motifs occurring at or near the cleavage site of the strand. This antisense strand may also contain one or more wing modifications in an alignment similar to the wing modifications that may be present on the sense strand.

In one embodiment, the wing modification on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two terminal nucleotides at the 3'-end, 5'-end or both ends of the strand.

In another embodiment, the wing modification on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two paired nucleotides within the duplex region at the 3'-end, 5'-end or both ends of the strand.

When the sense strand and the antisense strand of the RNAi agent each contain at least one wing modification, the wing modifications may fall on the same end of the duplex region, and have an overlap of one, two or three nucleotides.

When the sense strand and the antisense strand of the RNAi agent each contain at least two wing modifications, the sense strand and the antisense strand can be so aligned that two modifications each from one strand fall on one end of the duplex region, having an overlap of one, two or three nucleotides; two

modifications each from one strand fall on the other end of the duplex region, having an overlap of one, two or three nucleotides; two modifications one strand fall on each side of the lead motif, having an overlap of one, two or three nucleotides in the duplex region.

In one embodiment, every nucleotide in the sense strand and antisense strand of the RNAi agent, including the nucleotides that are part of the motifs, may be modified. Each nucleotide may be modified with the same or different modification which can include one or more alteration of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens; alteration of a constituent of the ribose sugar, *e.g.*, of the 2' hydroxyl on the ribose sugar; wholesale replacement of the phosphate moiety with "dephospho" linkers; modification or replacement of a naturally occurring base; and replacement or
 10 modification of the ribose-phosphate backbone.

As nucleic acids are polymers of subunits, many of the modifications occur at a position which is repeated within a nucleic acid, *e.g.*, a modification of a base, or a phosphate moiety, or a non-linking O of a phosphate moiety. In some cases, the modification will occur at all of the subject positions in the nucleic acid but in many cases it will not. By way of example, a modification may only occur at a 3' or
 15 5' terminal position, may only occur in a terminal region, *e.g.*, at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand. A modification may occur in a double strand region, a single strand region, or in both. A modification may occur only in the double strand region of a RNA or may only occur in a single strand region of a RNA. For example, a phosphorothioate modification at a non-linking O position may only occur at one or both termini, may only occur in a terminal region, *e.g.*, at
 20 a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand, or may occur in double strand and single strand regions, particularly at termini. The 5' end or ends can be phosphorylated.

It may be possible, *e.g.*, to enhance stability, to include particular bases in overhangs, or to include modified nucleotides or nucleotide surrogates, in single strand overhangs, *e.g.*, in a 5' or 3'
 25 overhang, or in both. For example, it can be desirable to include purine nucleotides in overhangs. In some embodiments all or some of the bases in a 3' or 5' overhang may be modified, *e.g.*, with a modification described herein. Modifications can include, *e.g.*, the use of modifications at the 2' position of the ribose sugar with modifications that are known in the art, *e.g.*, the use of deoxyribonucleotides, 2'-deoxy-2'-fluoro (2'-F) or 2'-O-methyl modified instead of the ribosugar of the nucleobase, and
 30 modifications in the phosphate group, *e.g.*, phosphorothioate modifications. Overhangs need not be homologous with the target sequence.

In one embodiment, each residue of the sense strand and antisense strand is independently modified with LNA, CRN, cET, UNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-deoxy, 2'-hydroxyl, or 2'-fluoro. The strands can contain more than one modification. In one embodiment, each

residue of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. The term "HNA" refers to hexitol or hexose nucleic acid.

At least two different modifications are typically present on the sense strand and antisense strand. Those two modifications may be the 2'-O-methyl or 2'-fluoro modifications, or others.

In one embodiment, the N_a and/or N_b comprise modifications of an alternating pattern. The term "alternating motif" as used herein refers to a motif having one or more modifications, each modification occurring on alternating nucleotides of one strand. The alternating nucleotide may refer to one per every other nucleotide or one per every three nucleotides, or a similar pattern. For example, if A, B and C each represent one type of modification to the nucleotide, the alternating motif can be "ABABABABABAB...",

"AABBAABBAABB...", "AABAABAABAAB...", "AAABAAABAAAB...", "AAABBBAAABBB...", or "ABCABCABCABC...", etc.

The type of modifications contained in the alternating motif may be the same or different. For example, if A, B, C, D each represent one type of modification on the nucleotide, the alternating pattern, *i.e.*, modifications on every other nucleotide, may be the same, but each of the sense strand or antisense strand can be selected from several possibilities of modifications within the alternating motif such as "ABABAB...", "ACACAC..." "BDBDBD..." or "CDCDCD...", etc.

In one embodiment, the RNAi agent of the invention comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the alternating motif on the antisense strand is shifted. The shift may be such that the modified group of nucleotides of the sense strand corresponds to a differently modified group of nucleotides of the antisense strand and *vice versa*. For example, the sense strand when paired with the antisense strand in the dsRNA duplex, the alternating motif in the sense strand may start with "ABABAB" from 5'-3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 5'-3' of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5'-3' of the strand and the alternating motif in the antisense strand may start with "BBAABBAA" from 5'-3' of the strand within the duplex region, so that there is a complete or partial shift of the modification patterns between the sense strand and the antisense strand.

In one embodiment, the RNAi agent comprises the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the sense strand initially has a shift relative to the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the antisense strand initially, *i.e.*, the 2'-O-methyl modified nucleotide on the sense strand base pairs with a 2'-F modified nucleotide on the antisense strand and *vice versa*. The 1 position of the sense strand may start with the 2'-F modification, and the 1 position of the antisense strand may start with the 2'-O-methyl modification.

The introduction of one or more motifs of three identical modifications on three consecutive nucleotides to the sense strand and/or antisense strand interrupts the initial modification pattern present in the

sense strand and/or antisense strand. This interruption of the modification pattern of the sense and/or antisense strand by introducing one or more motifs of three identical modifications on three consecutive nucleotides to the sense and/or antisense strand surprisingly enhances the gene silencing activity to the target gene.

5 In one embodiment, when the motif of three identical modifications on three consecutive nucleotides is introduced to any of the strands, the modification of the nucleotide next to the motif is a different modification than the modification of the motif. For example, the portion of the sequence containing the motif is "...N_aYYYN_b..." where "Y" represents the modification of the motif of three identical modifications on three consecutive nucleotide, and "N_a" and "N_b" represent a modification to the nucleotide next to the motif
10 "YYY" that is different than the modification of Y, and where N_a and N_b can be the same or different modifications. Alternatively, N_a and/or N_b may be present or absent when there is a wing modification present.

The RNAi agent may further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage. The phosphorothioate or methylphosphonate internucleotide linkage modification may occur on any nucleotide of the sense strand or antisense strand or both strands in any position of the
15 strand. For instance, the internucleotide linkage modification may occur on every nucleotide on the sense strand and/or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand and/or antisense strand; or the sense strand or antisense strand may contain both internucleotide linkage modifications in an alternating pattern. The alternating pattern of the internucleotide linkage modification on the sense strand may be the same or different from the antisense strand, and the
20 alternating pattern of the internucleotide linkage modification on the sense strand may have a shift relative to the alternating pattern of the internucleotide linkage modification on the antisense strand. In one embodiment, a double-stranded RNAi agent comprises 6-8 phosphorothioate internucleotide linkages. In one embodiment, the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and the sense strand comprises at least two
25 phosphorothioate internucleotide linkages at either the 5'-terminus or the 3'-terminus.

In one embodiment, the RNAi comprises a phosphorothioate or methylphosphonate internucleotide linkage modification in the overhang region. For example, the overhang region may contain two nucleotides having a phosphorothioate or methylphosphonate internucleotide linkage between the two nucleotides. Internucleotide linkage modifications also may be made to link the overhang nucleotides with the terminal
30 paired nucleotides within the duplex region. For example, at least 2, 3, 4, or all the overhang nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage, and optionally, there may be additional phosphorothioate or methylphosphonate internucleotide linkages linking the overhang nucleotide with a paired nucleotide that is next to the overhang nucleotide. For instance, there may be at least two phosphorothioate internucleotide linkages between the terminal three nucleotides, in which two of the three
35 nucleotides are overhang nucleotides, and the third is a paired nucleotide next to the overhang nucleotide.

These terminal three nucleotides may be at the 3'-end of the antisense strand, the 3'-end of the sense strand, the 5'-end of the antisense strand, and/or the 5'-end of the antisense strand.

In one embodiment, the 2 nucleotide overhang is at the 3'-end of the antisense strand, and there are two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. Optionally, the RNAi agent may additionally have two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand.

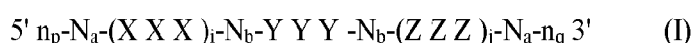
In one embodiment, the RNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mismatch may occur in the overhang region or the duplex region. The base pair may be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

In one embodiment, the RNAi agent comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'-end of the antisense strand independently selected from the group of: A:U, G:U, I:C, and mismatched pairs, *e.g.*, non-canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

In one embodiment, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from the group consisting of A, dA, dU, U, and dT. Alternatively, at least one of the first 1, 2 or 3 base pair within the duplex region from the 5'-end of the antisense strand is an AU base pair. For example, the first base pair within the duplex region from the 5'-end of the antisense strand is an AU base pair.

In another embodiment, the nucleotide at the 3'-end of the sense strand is deoxy-thymine (dT). In another embodiment, the nucleotide at the 3'-end of the antisense strand is deoxy-thymine (dT). In one embodiment, there is a short sequence of deoxy-thymine nucleotides, for example, two dT nucleotides on the 3'-end of the sense and/or antisense strand.

In one embodiment, the sense strand sequence may be represented by formula (I):



wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

each N_a independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

5 each n_p and n_q independently represent an overhang nucleotide;

wherein N_b and Y do not have the same modification; and

XXX , YYY and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. Preferably YYY is all 2'-F modified nucleotides.

In one embodiment, the N_a and/or N_b comprise modifications of alternating pattern.

10 In one embodiment, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (*e.g.*: can occur at positions 6, 7, 8, 7, 8, 9, 8, 9, 10, 9, 10, 11, 10, 11, 12 or 11, 12, 13) of the sense strand, the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'-end.

15 In one embodiment, i is 1 and j is 0, or i is 0 and j is 1, or both i and j are 1. The sense strand can therefore be represented by the following formulas:

5' n_p - N_a - YYY - N_b - ZZZ - N_a - n_q 3' (Ib);

5' n_p - N_a - XXX - N_b - YYY - N_a - n_q 3' (Ic); or

5' n_p - N_a - XXX - N_b - YYY - N_b - ZZZ - N_a - n_q 3' (Id).

20 When the sense strand is represented by formula (Ib), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Ic), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a can independently represent
25 an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Id), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5 or 6. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

30 Each of X , Y and Z may be the same or different from each other.

In other embodiments, i is 0 and j is 0, and the sense strand may be represented by the formula:

5' n_p - N_a - YYY - N_a - n_q 3' (Ia).

When the sense strand is represented by formula (Ia), each N_a independently can represent an
35 oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

In one embodiment, the antisense strand sequence of the RNAi may be represented by formula (Ie):



wherein:

k and l are each independently 0 or 1;

5 p' and q' are each independently 0-6;

each N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

10 each n_p' and n_q' independently represent an overhang nucleotide;

wherein N_b' and Y' do not have the same modification; and

X'X'X', Y'Y'Y' and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

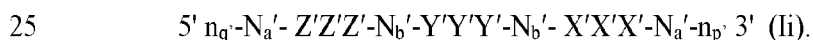
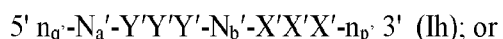
In one embodiment, the N_a' and/or N_b' comprise modifications of alternating pattern.

15 The Y'Y'Y' motif occurs at or near the cleavage site of the antisense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleotide in length, the Y'Y'Y' motif can occur at positions 9, 10, 11; 10, 11, 12; 11, 12, 13; 12, 13, 14; or 13, 14, 15 of the antisense strand, with the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'-end. Preferably, the Y'Y'Y' motif occurs at positions 11, 12, 13.

20 In one embodiment, Y'Y'Y' motif is all 2'-OMe modified nucleotides.

In one embodiment, k is 1 and l is 0, or k is 0 and l is 1, or both k and l are 1.

The antisense strand can therefore be represented by the following formulas:



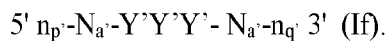
When the antisense strand is represented by formula (Ig), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

30 When the antisense strand is represented as formula (Ih), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (Ii), each N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

35 Preferably, N_b is 0, 1, 2, 3, 4, 5 or 6.

In other embodiments, k is 0 and l is 0 and the antisense strand may be represented by the formula:



When the antisense strand is represented as formula (If), each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

Each of X' , Y' and Z' may be the same or different from each other.

Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, CRN, UNA, cEt, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-hydroxyl, or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. Each X , Y , Z , X' , Y' and Z' , in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

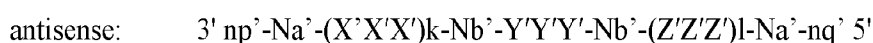
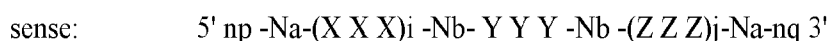
In one embodiment, the sense strand of the RNAi agent may contain YYY motif occurring at 9, 10 and 11 positions of the strand when the duplex region is 21 nt, the count starting from the 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'-end; and Y represents 2'-F modification. The sense strand may additionally contain XXX motif or ZZZ motifs as wing modifications at the opposite end of the duplex region; and XXX and ZZZ each independently represents a 2'-OMe modification or 2'-F modification.

In one embodiment the antisense strand may contain $Y'Y'Y'$ motif occurring at positions 11, 12, 13 of the strand, the count starting from the 1st nucleotide from the 5' end, or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'-end; and Y' represents 2'-O-methyl modification.

The antisense strand may additionally contain $X'X'X'$ motif or $Z'Z'Z'$ motifs as wing modifications at the opposite end of the duplex region; and $X'X'X'$ and $Z'Z'Z'$ each independently represents a 2'-OMe modification or 2'-F modification.

The sense strand represented by any one of the above formulas (Ia), (Ib), (Ic), and (Id) forms a duplex with an antisense strand being represented by any one of formulas (If), (Ig), (Ih), and (Ii), respectively.

Accordingly, the RNAi agents for use in the methods of the invention may comprise a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, the RNAi duplex represented by formula (Ij):



(Ij)

wherein:

i , j , k , and l are each independently 0 or 1;

p , p' , q , and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each Nb and Nb' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

wherein each np', np, nq', and nq, each of which may or may not be present, independently represents an overhang nucleotide; and

5 XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one embodiment, i is 0 and j is 0; or i is 1 and j is 0; or i is 0 and j is 1; or both i and j are 0; or both i and j are 1. In another embodiment, k is 0 and l is 0; or k is 1 and l is 0; k is 0 and l is 1; or both k and l are 0; or both k and l are 1.

10 Exemplary combinations of the sense strand and antisense strand forming a RNAi duplex include the formulas below:

5' np - Na -Y Y Y -Na-nq 3'

3' np'-Na'-Y'Y'Y' -Na'nq' 5' (Ik)

5' np -Na -Y Y Y -Nb -Z Z Z -Na-nq 3'

15 3' np'-Na'-Y'Y'Y'-Nb'-Z'Z'Z'-Na'nq' 5' (II)

5' np-Na- X X X -Nb -Y Y Y - Na-nq 3'

3' np'-Na'-X'X'X'-Nb'-Y'Y'Y'-Na'nq' 5' (Im)

5' np -Na -X X X -Nb-Y Y Y -Nb- Z Z Z -Na-nq 3'

3' np'-Na'-X'X'X'-Nb'-Y'Y'Y'-Nb'-Z'Z'Z'-Na-nq' 5' (In)

20 When the RNAi agent is represented by formula (Ik), each Na independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the RNAi agent is represented by formula (II), each Nb independently represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5 or 1-4 modified nucleotides. Each Na independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

25 When the RNAi agent is represented as formula (Im), each Nb, Nb' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each Na independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the RNAi agent is represented as formula (In), each Nb, Nb' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each Na, Na' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of Na, Na', Nb and Nb' independently comprises modifications of alternating pattern.

Each of X, Y and Z in formulas (Ij), (Ik), (II), (Im), and (In) may be the same or different from each other.

When the RNAi agent is represented by formula (Ij), (Ik), (II), (Im), and (In), at least one of the Y nucleotides may form a base pair with one of the Y' nucleotides. Alternatively, at least two of the Y

nucleotides form base pairs with the corresponding Y' nucleotides; or all three of the Y nucleotides all form base pairs with the corresponding Y' nucleotides.

When the RNAi agent is represented by formula (II) or (In), at least one of the Z nucleotides may form a base pair with one of the Z' nucleotides. Alternatively, at least two of the Z nucleotides form base pairs with the corresponding Z' nucleotides; or all three of the Z nucleotides all form base pairs with the corresponding Z' nucleotides.

When the RNAi agent is represented as formula (Im) or (In), at least one of the X nucleotides may form a base pair with one of the X' nucleotides. Alternatively, at least two of the X nucleotides form base pairs with the corresponding X' nucleotides; or all three of the X nucleotides all form base pairs with the corresponding X' nucleotides.

In one embodiment, the modification on the Y nucleotide is different than the modification on the Y' nucleotide, the modification on the Z nucleotide is different than the modification on the Z' nucleotide, and/or the modification on the X nucleotide is different than the modification on the X' nucleotide.

In one embodiment, when the RNAi agent is represented by formula (In), the Na modifications are 2'-O-methyl or 2'-fluoro modifications. In another embodiment, when the RNAi agent is represented by formula (In), the Na modifications are 2'-O-methyl or 2'-fluoro modifications and $np' > 0$ and at least one np' is linked to a neighboring nucleotide via a phosphorothioate linkage. In yet another embodiment, when the RNAi agent is represented by formula (In), the Na modifications are 2'-O-methyl or 2'-fluoro modifications, $np' > 0$ and at least one np' is linked to a neighboring nucleotide via phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker (described below). In another embodiment, when the RNAi agent is represented by formula (In), the Na modifications are 2'-O-methyl or 2'-fluoro modifications, $np' > 0$ and at least one np' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, when the RNAi agent is represented by formula (Ik), the Na modifications are 2'-O-methyl or 2'-fluoro modifications, $np' > 0$ and at least one np' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, the RNAi agent is a multimer containing at least two duplexes represented by formula (Ij), (Ik), (Il), (Im), and (In), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can

target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In one embodiment, the RNAi agent is a multimer containing three, four, five, six or more duplexes represented by formula (Ij), (Ik), (Il), (Im), and (In), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

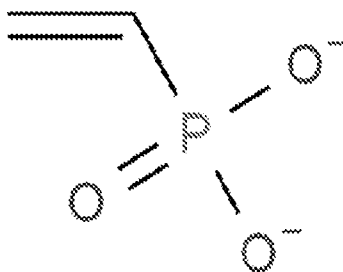
In one embodiment, two RNAi agents represented by formula (Ij), (Ik), (Il), (Im), and (In) are linked to each other at the 5' end, and one or both of the 3' ends and are optionally conjugated to a ligand. Each of the agents can target the same gene or two different genes; or each of the agents can target same gene at two different target sites.

In certain embodiments, an RNAi agent of the invention may contain a low number of nucleotides containing a 2'-fluoro modification, e.g., 10 or fewer nucleotides with 2'-fluoro modification. For example, the RNAi agent may contain 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotides with a 2'-fluoro modification. In a specific embodiment, the RNAi agent of the invention contains 10 nucleotides with a 2'-fluoro modification, e.g., 4 nucleotides with a 2'-fluoro modification in the sense strand and 6 nucleotides with a 2'-fluoro modification in the antisense strand. In another specific embodiment, the RNAi agent of the invention contains 6 nucleotides with a 2'-fluoro modification, e.g., 4 nucleotides with a 2'-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

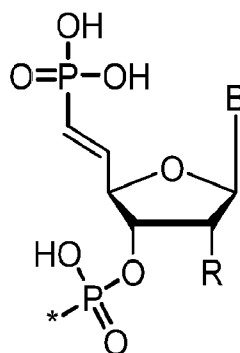
In other embodiments, an RNAi agent of the invention may contain an ultra-low number of nucleotides containing a 2'-fluoro modification, e.g., 2 or fewer nucleotides containing a 2'-fluoro modification. For example, the RNAi agent may contain 2, 1 or 0 nucleotides with a 2'-fluoro modification. In a specific embodiment, the RNAi agent may contain 2 nucleotides with a 2'-fluoro modification, e.g., 0 nucleotides with a 2'-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

Various publications describe multimeric RNAi agents that can be used in the methods of the invention. Such publications include WO2007/091269, US Patent No. 7858769, WO2010/141511, WO2007/117686, WO2009/014887 and WO2011/031520 the entire contents of each of which are hereby incorporated herein by reference.

In certain embodiments, the compositions and methods of the disclosure include a vinyl phosphonate (VP) modification of an RNAi agent as described herein. In exemplary embodiments, a vinyl phosphonate of the disclosure has the following structure:



For example, when the phosphate mimic is a 5'-vinyl phosphonate (VP), the 5'-terminal nucleotide can have the following structure,



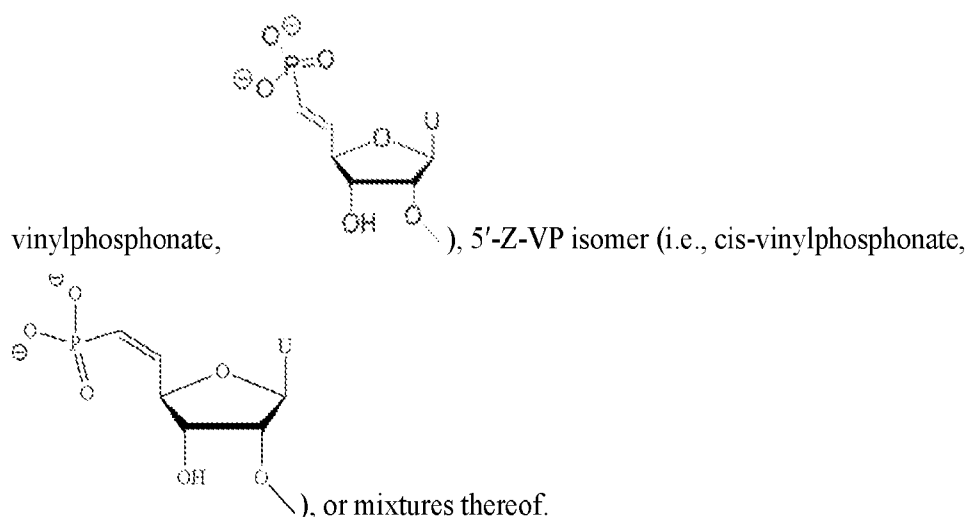
5 wherein * indicates the location of the bond to 5'-position of the adjacent nucleotide;

R is hydrogen, hydroxy, methoxy, fluoro (e.g., hydroxy or methoxy), or another modification described herein; and

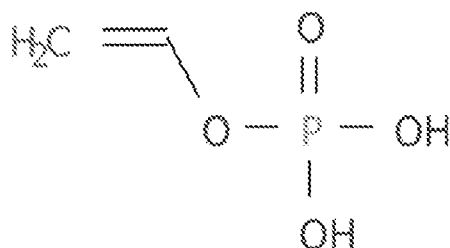
B is a nucleobase or a modified nucleobase, optionally where B is adenine, guanine, cytosine, thymine or uracil.

10 A vinyl phosphonate of the instant disclosure may be attached to either the antisense or the sense strand of a dsRNA of the disclosure. In certain embodiments, a vinyl phosphonate of the instant disclosure is attached to the antisense strand of a dsRNA, optionally at the 5' end of the antisense strand of the dsRNA. The dsRNA agent can comprise a phosphorus-containing group at the 5'-end of the sense strand or antisense strand. The 5'-end phosphorus-containing group can be 5'-end phosphate (5'-P), 5'-end phosphorothioate (5'-PS), 5'-end phosphorodithioate (5'-PS2), 5'-end vinylphosphonate (5'-VP), 5'-end methylphosphonate (MePhos), or 5'-deoxy-5'-C-malonyl. When the 5'-end phosphorus-containing group is 5'-end vinylphosphonate (5'-VP), the 5'-VP can be either 5'-E-VP isomer (i.e., trans-

15



Vinyl phosphate modifications are also contemplated for the compositions and methods of the instant disclosure. An exemplary vinyl phosphate structure is:



For example, when the phosphate mimic is a 5'-vinyl phosphate, the 5'-terminal nucleotide can have the immediately structure, where the phosphonate group is replaced by a phosphate.

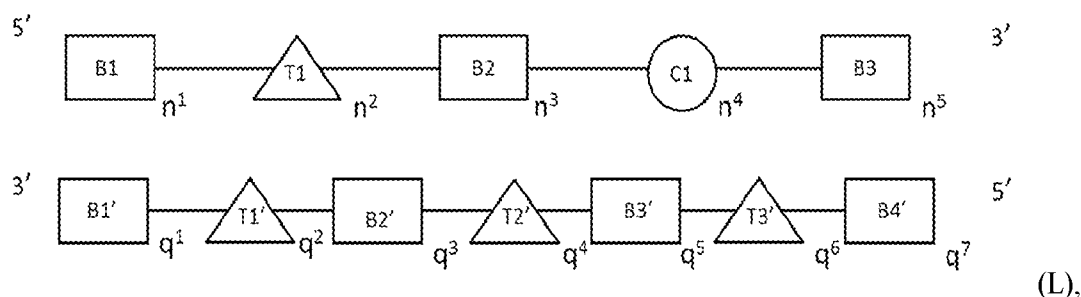
As described in more detail below, the RNAi agent that contains conjugations of one or more carbohydrate moieties to a RNAi agent can optimize one or more properties of the RNAi agent. In many cases, the carbohydrate moiety will be attached to a modified subunit of the RNAi agent. For example, the ribose sugar of one or more ribonucleotide subunits of a dsRNA agent can be replaced with another moiety, *e.g.*, a non-carbohydrate (preferably cyclic) carrier to which is attached a carbohydrate ligand. A ribonucleotide subunit in which the ribose sugar of the subunit has been so replaced is referred to herein as a ribose replacement modification subunit (RRMS). A cyclic carrier may be a carbocyclic ring system, *i.e.*, all ring atoms are carbon atoms, or a heterocyclic ring system, *i.e.*, one or more ring atoms may be a heteroatom, *e.g.*, nitrogen, oxygen, sulfur. The cyclic carrier may be a monocyclic ring system, or may contain two or more rings, *e.g.* fused rings. The cyclic carrier may be a fully saturated ring system, or it may contain one or more double bonds.

The ligand may be attached to the polynucleotide via a carrier. The carriers include (i) at least one “backbone attachment point,” preferably two “backbone attachment points” and (ii) at least one “tethering attachment point.” A “backbone attachment point” as used herein refers to a functional group, *e.g.* a hydroxyl group, or generally, a bond available for, and that is suitable for incorporation of the carrier into the backbone, *e.g.*, the phosphate, or modified phosphate, *e.g.*, sulfur containing, backbone, of a ribonucleic acid. A

“tethering attachment point” (TAP) in some embodiments refers to a constituent ring atom of the cyclic carrier, e.g., a carbon atom or a heteroatom (distinct from an atom which provides a backbone attachment point), that connects a selected moiety. The moiety can be, e.g., a carbohydrate, e.g. monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide and polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic carrier will often include a functional group, e.g., an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, e.g., a ligand to the constituent ring.

The RNAi agents may be conjugated to a ligand *via* a carrier, wherein the carrier can be cyclic group or acyclic group; preferably, the cyclic group is selected from pyrrolidiny, pyrazoliny, pyrazolidiny, imidazoliny, imidazolidiny, piperidiny, piperaziny, [1,3]dioxolane, oxazolidiny, isoxazolidiny, morpholiny, thiazolidiny, isothiazolidiny, quinoxaliny, pyridazinonyl, tetrahydrofuryl and decalin; preferably, the acyclic group is selected from serinol backbone or diethanolamine backbone.

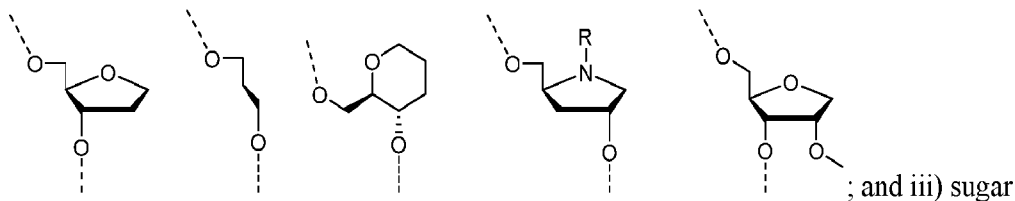
In another embodiment of the invention, an iRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides. The RNAi agent may be represented by formula (L):



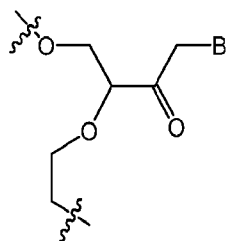
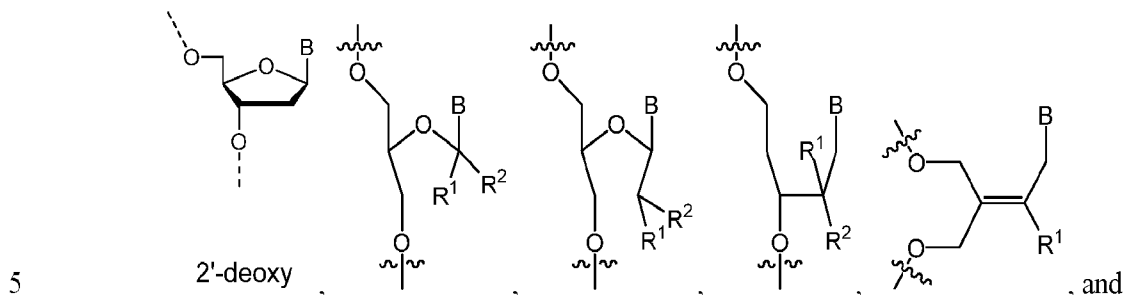
In formula (L), B1, B2, B3, B1', B2', B3', and B4' each are independently a nucleotide containing a modification selected from the group consisting of 2'-O-alkyl, 2'-substituted alkoxy, 2'-substituted alkyl, 2'-halo, ENA, and BNA/LNA. In certain embodiments, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe modifications. In certain embodiments, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe or 2'-F modifications. In certain embodiments, at least one of B1, B2, B3, B1', B2', B3', and B4' contain 2'-O-N-methylacetamido (2'-O-NMA) modification.

C1 is a thermally destabilizing nucleotide placed at a site opposite to the seed region of the antisense strand (i.e., at positions 2-8 of the 5'-end of the antisense strand). For example, C1 is at a position of the sense strand that pairs with a nucleotide at positions 2-8 of the 5'-end of the antisense strand. In one example, C1 is at position 15 from the 5'-end of the sense strand. C1 nucleotide bears the thermally destabilizing modification which can include abasic modification; mismatch with the opposing nucleotide in the duplex; and sugar modification such as 2'-deoxy modification or acyclic nucleotide e.g., unlocked nucleic acids (UNA) or glycerol nucleic acid (GNA). In certain embodiments, C1 has thermally

destabilizing modification selected from the group consisting of: i) mismatch with the opposing nucleotide in the antisense strand; ii) abasic modification selected from the group consisting of:

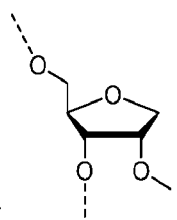


modification selected from the group consisting of:



, wherein B is a modified or unmodified nucleobase, R^1 and R^2 independently are H,

halogen, OR_3 , or alkyl; and R_3 is H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar. In certain embodiments, the thermally destabilizing modification in C1 is a mismatch selected from the group consisting of G:G, G:A, G:U, G:T, A:A, A:C, C:C, C:U, C:T, U:U, T:T, and U:T; and optionally, at least one nucleobase in the mismatch pair is a 2'-deoxy nucleobase. In one example, the thermally



destabilizing modification in C1 is GNA or

T1, T1', T2', and T3' each independently represent a nucleotide comprising a modification providing the nucleotide a steric bulk that is less or equal to the steric bulk of a 2'-OMe modification. A steric bulk refers to the sum of steric effects of a modification. Methods for determining steric effects of a modification of a nucleotide are known to one skilled in the art. The modification can be at the 2' position of a ribose sugar of the nucleotide, or a modification to a non-ribose nucleotide, acyclic nucleotide, or the backbone of the nucleotide that is similar or equivalent to the 2' position of the ribose sugar, and provides the nucleotide a steric bulk that is less than or equal to the steric bulk of a 2'-OMe modification. For example, T1, T1', T2', and T3' are each independently selected from DNA, RNA, LNA, 2'-F, and 2'-F-5'-methyl. In certain embodiments, T1 is DNA. In certain embodiments, T1' is

DNA, RNA or LNA. In certain embodiments, T2' is DNA or RNA. In certain embodiments, T3' is DNA or RNA.

n^1 , n^3 , and q^1 are independently 4 to 15 nucleotides in length.

n^5 , q^3 , and q^7 are independently 1-6 nucleotide(s) in length.

5 n^4 , q^2 , and q^6 are independently 1-3 nucleotide(s) in length; alternatively, n^4 is 0.

q^5 is independently 0-10 nucleotide(s) in length.

n^2 and q^4 are independently 0-3 nucleotide(s) in length.

Alternatively, n^4 is 0-3 nucleotide(s) in length.

10 In certain embodiments, n^4 can be 0. In one example, n^4 is 0, and q^2 and q^6 are 1. In another example, n^4 is 0, and q^2 and q^6 are 1, with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

15 In certain embodiments, n^4 , q^2 , and q^6 are each 1.

In certain embodiments, n^2 , n^4 , q^2 , q^4 , and q^6 are each 1.

In certain embodiments, C1 is at position 14-17 of the 5'-end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^4 is 1. In certain embodiments, C1 is at position 15 of the 5'-end of the sense strand

20 In certain embodiments, T3' starts at position 2 from the 5' end of the antisense strand. In one example, T3' is at position 2 from the 5' end of the antisense strand and q^6 is equal to 1.

In certain embodiments, T1' starts at position 14 from the 5' end of the antisense strand. In one example, T1' is at position 14 from the 5' end of the antisense strand and q^2 is equal to 1.

25 In an exemplary embodiment, T3' starts from position 2 from the 5' end of the antisense strand and T1' starts from position 14 from the 5' end of the antisense strand. In one example, T3' starts from position 2 from the 5' end of the antisense strand and q^6 is equal to 1 and T1' starts from position 14 from the 5' end of the antisense strand and q^2 is equal to 1.

In certain embodiments, T1' and T3' are separated by 11 nucleotides in length (*i.e.* not counting the T1' and T3' nucleotides).

30 In certain embodiments, T1' is at position 14 from the 5' end of the antisense strand. In one example, T1' is at position 14 from the 5' end of the antisense strand and q^2 is equal to 1, and the modification at the 2' position or positions in a non-ribose, acyclic or backbone that provide less steric bulk than a 2'-OMe ribose.

35 In certain embodiments, T3' is at position 2 from the 5' end of the antisense strand. In one example, T3' is at position 2 from the 5' end of the antisense strand and q^6 is equal to 1, and the

modification at the 2' position or positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

In certain embodiments, T1 is at the cleavage site of the sense strand. In one example, T1 is at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1. In an exemplary embodiment, T1 is at the cleavage site of the sense strand at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1,

In certain embodiments, T2' starts at position 6 from the 5' end of the antisense strand. In one example, T2' is at positions 6-10 from the 5' end of the antisense strand, and q^4 is 1.

In an exemplary embodiment, T1 is at the cleavage site of the sense strand, for instance, at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1; T1' is at position 14 from the 5' end of the antisense strand, and q^2 is equal to 1, and the modification to T1' is at the 2' position of a ribose sugar or at positions in a non-ribose, acyclic or backbone that provide less steric bulk than a 2'-OMe ribose; T2' is at positions 6-10 from the 5' end of the antisense strand, and q^4 is 1; and T3' is at position 2 from the 5' end of the antisense strand, and q^6 is equal to 1, and the modification to T3' is at the 2' position or at positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

In certain embodiments, T2' starts at position 8 from the 5' end of the antisense strand. In one example, T2' starts at position 8 from the 5' end of the antisense strand, and q^4 is 2.

In certain embodiments, T2' starts at position 9 from the 5' end of the antisense strand. In one example, T2' is at position 9 from the 5' end of the antisense strand, and q^4 is 1.

In certain embodiments, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In certain embodiments, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 6, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 7, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 6, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 7, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 1, B3' is 2'-OMe or 2'-F, q⁵ is 6, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 1, B3' is 2'-OMe or 2'-F, q⁵ is 6, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 5, T2' is 2'-F, q⁴ is 1, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 5, T2' is 2'-F, q⁴ is 1, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1.

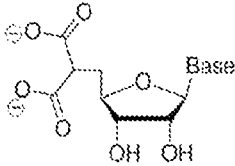
In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

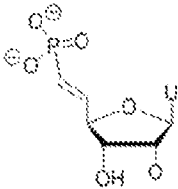
In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1.

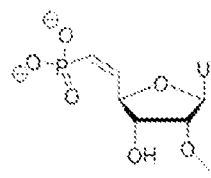
In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting

from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

The RNAi agent can comprise a phosphorus-containing group at the 5'-end of the sense strand or antisense strand. The 5'-end phosphorus-containing group can be 5'-end phosphate (5'-P), 5'-end phosphorothioate (5'-PS), 5'-end phosphorodithioate (5'-PS₂), 5'-end vinylphosphonate (5'-VP), 5'-end

methylphosphonate (MePhos), or 5'-deoxy-5'-C-malonyl (). When the 5'-end phosphorus-containing group is 5'-end vinylphosphonate (5'-VP), the 5'-VP can be either 5'-E-VP

isomer (i.e., *trans*-vinylphosphonate, ), 5'-Z-VP isomer (i.e., *cis*-vinylphosphonate,



), or mixtures thereof.

In certain embodiments, the RNAi agent comprises a phosphorus-containing group at the 5'-end of the sense strand. In certain embodiments, the RNAi agent comprises a phosphorus-containing group at the 5'-end of the antisense strand.

In certain embodiments, the RNAi agent comprises a 5'-P. In certain embodiments, the RNAi agent comprises a 5'-P in the antisense strand.

In certain embodiments, the RNAi agent comprises a 5'-PS. In certain embodiments, the RNAi agent comprises a 5'-PS in the antisense strand.

In certain embodiments, the RNAi agent comprises a 5'-VP. In certain embodiments, the RNAi agent comprises a 5'-VP in the antisense strand. In certain embodiments, the RNAi agent comprises a 5'-E-VP in the antisense strand. In certain embodiments, the RNAi agent comprises a 5'-Z-VP in the antisense strand.

In certain embodiments, the RNAi agent comprises a 5'-PS₂. In certain embodiments, the RNAi agent comprises a 5'-PS₂ in the antisense strand.

In certain embodiments, the RNAi agent comprises a 5'-PS₂. In certain embodiments, the RNAi agent comprises a 5'-deoxy-5'-C-malonyl in the antisense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

5 In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-P.

10 In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

15 In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-PS₂.

20 In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

25 In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P.

30 In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within

positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS₂.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-P.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The dsRNA agent also comprises a 5'-PS.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

5 In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-PS₂.

10 In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

15 In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-P.

20 In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS.

25 In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS₂.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-P.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The dsRNA RNA agent also comprises a 5'-PS₂.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS₂.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two

phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'-P.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'-PS.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'-PS₂.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is

4, q^4 is 0, $B3'$ is 2'-OMe or 2'-F, q^5 is 7, $T3'$ is 2'-F, q^6 is 1, $B4'$ is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS.

In certain embodiments, $B1$ is 2'-OMe or 2'-F, n^1 is 8, $T1$ is 2'F, n^2 is 3, $B2$ is 2'-OMe, n^3 is 7, n^4 is 0, $B3$ is 2'-OMe, n^5 is 3, $B1'$ is 2'-OMe or 2'-F, q^1 is 9, $T1'$ is 2'-F, q^2 is 1, $B2'$ is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, $B3'$ is 2'-OMe or 2'-F, q^5 is 7, $T3'$ is 2'-F, q^6 is 1, $B4'$ is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In certain embodiments, $B1$ is 2'-OMe or 2'-F, n^1 is 8, $T1$ is 2'F, n^2 is 3, $B2$ is 2'-OMe, n^3 is 7, n^4 is 0, $B3$ is 2'-OMe, n^5 is 3, $B1'$ is 2'-OMe or 2'-F, q^1 is 9, $T1'$ is 2'-F, q^2 is 1, $B2'$ is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, $B3'$ is 2'-OMe or 2'-F, q^5 is 7, $T3'$ is 2'-F, q^6 is 1, $B4'$ is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS₂.

In certain embodiments, $B1$ is 2'-OMe or 2'-F, n^1 is 8, $T1$ is 2'F, n^2 is 3, $B2$ is 2'-OMe, n^3 is 7, n^4 is 0, $B3$ is 2'-OMe, n^5 is 3, $B1'$ is 2'-OMe or 2'-F, q^1 is 9, $T1'$ is 2'-F, q^2 is 1, $B2'$ is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, $B3'$ is 2'-OMe or 2'-F, q^5 is 7, $T3'$ is 2'-F, q^6 is 1, $B4'$ is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In certain embodiments, $B1$ is 2'-OMe or 2'-F, n^1 is 8, $T1$ is 2'F, n^2 is 3, $B2$ is 2'-OMe, n^3 is 7, n^4 is 0, $B3$ is 2'-OMe, n^5 is 3, $B1'$ is 2'-OMe or 2'-F, q^1 is 9, $T1'$ is 2'-F, q^2 is 1, $B2'$ is 2'-OMe or 2'-F, q^3 is 4, $T2'$ is 2'-F, q^4 is 2, $B3'$ is 2'-OMe or 2'-F, q^5 is 5, $T3'$ is 2'-F, q^6 is 1, $B4'$ is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage

modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In certain embodiments, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

5 In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage
10 modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS and a targeting ligand. In certain embodiments, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage
15 modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-E-VP, 5'-Z-VP, or combination thereof), and a targeting ligand.

In certain embodiments, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage
25 modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS₂ and a targeting ligand. In certain embodiments, the 5'-PS₂ is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with
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two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In certain embodiments, the 5'-deoxy-5'-C-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-P and a targeting ligand. In certain embodiments, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS and a targeting ligand. In certain embodiments, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-E-VP, 5'-Z-VP, or combination thereof) and a targeting ligand. In certain embodiments, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS₂ and a targeting ligand. In certain embodiments, the 5'-PS₂ is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In certain embodiments, the 5'-deoxy-5'-C-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In certain embodiments, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises

a 5'-PS and a targeting ligand. In certain embodiments, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP (e.g., a 5'-E-VP, 5'-Z-VP, or combination thereof) and a targeting ligand. In certain embodiments, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS₂ and a targeting ligand. In certain embodiments, the 5'-PS₂ is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In certain embodiments, the 5'-deoxy-5'-C-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at

positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In certain embodiments, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS and a targeting ligand. In certain embodiments, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-E-VP, 5'-Z-VP, or combination thereof) and a targeting ligand. In certain embodiments, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS₂ and a targeting ligand. In certain embodiments, the 5'-PS₂ is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In certain embodiments, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two

phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In certain embodiments, the 5'-deoxy-5'-C-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In a particular embodiment, an RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker; and

(iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14 to 16, 18, and 20 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 9, 11 to 13, 15, 17, 19, 21, and 23, and 2'-F modifications at positions 2, 4, 6 to 8, 10, 14, 16, 18, 20, and 22 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the dsRNA agents have a two-nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, an RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 15, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14, 16, 18, and 20 (counting from the 5' end);

and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

- (i) a length of 23 nucleotides;
- (ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and
- (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two-nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, an RNAi agent of the present invention comprises:

- (a) a sense strand having:
 - (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-OMe modifications at positions 1 to 6, 8, 10, and 12 to 21, 2'-F modifications at positions 7, and 9, and a desoxy-nucleotide (*e.g.* dT) at position 11 (counting from the 5' end); and
 - (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

- (b) an antisense strand having:
 - (i) a length of 23 nucleotides;
 - (ii) 2'-OMe modifications at positions 1, 3, 7, 9, 11, 13, 15, 17, and 19 to 23, and 2'-F modifications at positions 2, 4 to 6, 8, 10, 12, 14, 16, and 18 (counting from the 5' end); and
 - (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two-nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, an RNAi agent of the present invention comprises:

- (a) a sense strand having:
 - (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, 10, 12, 14, and 16 to 21, and 2'-F modifications at positions 7, 9, 11, 13, and 15; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

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(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 5, 7, 9, 11, 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2 to 4, 6, 8, 10, 12, 14, 16, 18, and 20 (counting from the 5' end); and

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(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two-nucleotide overhang at the 3'-end of the antisense strand,

15 and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, an RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

20

(iii) 2'-OMe modifications at positions 1 to 9, and 12 to 21, and 2'-F modifications at positions 10, and 11; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

25

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and

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(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two-nucleotide overhang at the 3'-end of the antisense strand,

35 and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

- (a) a sense strand having:
 - (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, and 13, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, and 14 to 21; and
 - (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);
 - and
 - (b) an antisense strand having:
 - (i) a length of 23 nucleotides;
 - (ii) 2'-OMe modifications at positions 1, 3, 5 to 7, 9, 11 to 13, 15, 17 to 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 8, 10, 14, 16, and 20 (counting from the 5' end); and
 - (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);
- wherein the RNAi agents have a two-nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, an RNAi agent of the present invention comprises:

- (a) a sense strand having:
 - (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-OMe modifications at positions 1, 2, 4, 6, 8, 12, 14, 15, 17, and 19 to 21, and 2'-F modifications at positions 3, 5, 7, 9 to 11, 13, 16, and 18; and
 - (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);
- and
- (b) an antisense strand having:
 - (i) a length of 25 nucleotides;
 - (ii) 2'-OMe modifications at positions 1, 4, 6, 7, 9, 11 to 13, 15, 17, and 19 to 23, 2'-F modifications at positions 2, 3, 5, 8, 10, 14, 16, and 18, and desoxy-nucleotides (*e.g.* dT) at positions 24 and 25 (counting from the 5' end); and

- (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a four-nucleotide overhang at the 3'-end of the antisense strand,

5 and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

- (i) a length of 21 nucleotides;
- (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises
10 three GalNAc derivatives attached through a trivalent branched linker;
- (iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and
- (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

15 and

(b) an antisense strand having:

- (i) a length of 23 nucleotides;
- (ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 8, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 9, 14, and 16 (counting from the 5' end); and
20
- (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two-nucleotide overhang at the 3'-end of the antisense strand,
and a blunt end at the 5'-end of the antisense strand.

25 In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

- (i) a length of 21 nucleotides;
- (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
- (iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and
30
- (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

35 (b) an antisense strand having:

- (i) a length of 23 nucleotides;
- (ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and
- (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two-nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

- (a) a sense strand having:

- (i) a length of 19 nucleotides;
- (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
- (iii) 2'-OMe modifications at positions 1 to 4, 6, and 10 to 19, and 2'-F modifications at positions 5, and 7 to 9; and
- (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

- (b) an antisense strand having:

- (i) a length of 21 nucleotides;
- (ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 21, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and
- (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 19 and 20, and between nucleotide positions 20 and 21 (counting from the 5' end);

wherein the RNAi agents have a two-nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In certain embodiments, the iRNA for use in the methods of the invention is an agent selected from agents listed in Tables 3-6. These agents may further comprise a ligand.

IV. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves chemically linking to the RNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the iRNA. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, (1989) *Proc. Natl. Acad. Sci. USA*, 86: 6553-6556), cholic acid (Manoharan *et al.*, (1994)

Biorg. Med. Chem. Let., 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, (1992) *Ann. N.Y. Acad. Sci.*, 660:306-309; Manoharan *et al.*, (1993) *Biorg. Med. Chem. Let.*, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, (1992) *Nucl. Acids Res.*, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, (1991) *EMBO J.*, 10:1111-1118; Kabanov *et al.*, (1990) *FEBS Lett.*, 259:327-330; Svinarchuk *et al.*, (1993) *Biochimie*, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan *et al.*, (1995) *Tetrahedron Lett.*, 36:3651-3654; Shea *et al.*, (1990) *Nucl. Acids Res.*, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, (1995) *Nucleosides & Nucleotides*, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, (1995) *Tetrahedron Lett.*, 36:3651-3654), a palmityl moiety (Mishra *et al.*, (1995) *Biochim. Biophys. Acta*, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al.*, (1996) *J. Pharmacol. Exp. Ther.*, 277:923-937).

In one embodiment, a ligand alters the distribution, targeting or lifetime of an iRNA agent into which it is incorporated. In preferred embodiments a ligand provides an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment, *e.g.*, a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent such a ligand. Preferred ligands will not take part in duplex pairing in a duplexed nucleic acid.

Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g.*, a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, N-acetylglucosamine, N-acetylgalactosamine or hyaluronic acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolide) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacrylic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

Ligands can also include targeting groups, *e.g.*, a cell or tissue targeting agent, *e.g.*, a lectin, glycoprotein, lipid or protein, *e.g.*, an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, vitamin A, biotin, or an RGD peptide or RGD peptide mimetic.

Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), lipophilic molecules, *e.g.*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine) and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, [MPEG]₂, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu³⁺ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a hepatic cell. Ligands can also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF- κ B.

The ligand can be a substance, *e.g.*, a drug, which can increase the uptake of the iRNA agent into the cell, for example, by disrupting the cell's cytoskeleton, *e.g.*, by disrupting the cell's microtubules, microfilaments, and/or intermediate filaments. The drug can be, for example, taxon, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins *etc.* Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin *etc.* Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). In addition, aptamers that bind serum components (*e.g.* serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

Ligand-conjugated oligonucleotides of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be reacted

directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

In the ligand-conjugated oligonucleotides and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

A. Lipid Conjugates

In one embodiment, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule preferably binds a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, neproxin or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, and/or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

A lipid based ligand can be used to inhibit, *e.g.*, control the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the body. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney.

In a preferred embodiment, the lipid based ligand binds HSA. Preferably, it binds HSA with a sufficient affinity such that the conjugate will be preferably distributed to a non-kidney tissue. However, it is preferred that the affinity not be so strong that the HSA-ligand binding cannot be reversed.

In another preferred embodiment, the lipid based ligand binds HSA weakly or not at all, such that the conjugate will be preferably distributed to the kidney. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid based ligand.

In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a target cell, *e.g.*, a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, *e.g.*, of the malignant or non-malignant type, *e.g.*, cancer cells. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include are B vitamin, *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by target cells such as liver cells. Also included are HSA and low density lipoprotein (LDL).

10 *B. Cell Permeation Agents*

In another aspect, the ligand is a cell-permeation agent, preferably a helical cell-permeation agent. Preferably, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopodia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. The helical agent is preferably an alpha-helical agent, which preferably has a lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or cross-linked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 3). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 4) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a “delivery” peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO: 5) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 6) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit for cell targeting purposes is an

arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

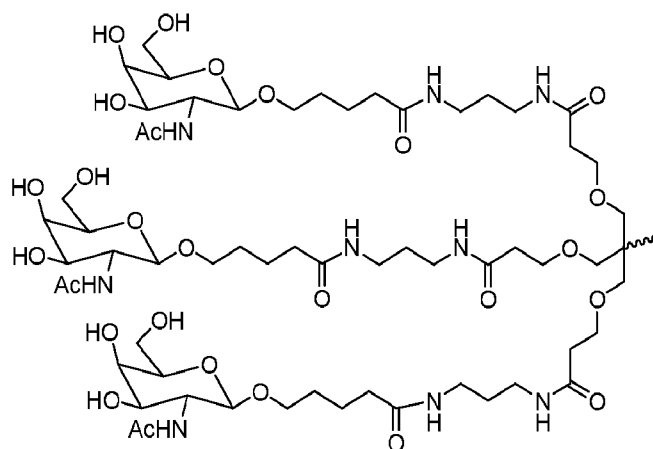
5 An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.*, glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidomimetics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Preferred conjugates of this ligand target PECAM-1 or VEGF.

10 A “cell permeation peptide” is capable of permeating a cell, *e.g.*, a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, a α -helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing peptide (*e.g.*, α -defensin, β -defensin or bactenecin), or a peptide containing only one or two dominating amino acids (*e.g.*, PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For
15 example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni *et al.*, Nucl. Acids Res. 31:2717-2724, 2003).

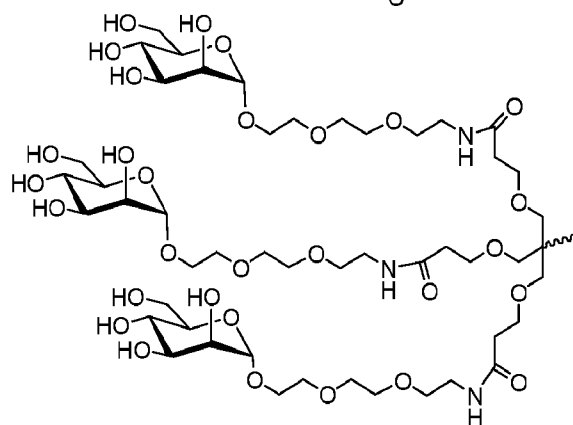
C. Carbohydrate Conjugates

In some embodiments of the compositions and methods of the invention, an iRNA oligonucleotide
20 further comprises a carbohydrate. The carbohydrate conjugated iRNA are advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, “carbohydrate” refers to a compound which is either a carbohydrate *per se* made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a
25 carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri- and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (*e.g.*, C5, C6, C7, or C8) sugars; di-
30 and trisaccharides include sugars having two or three monosaccharide units (*e.g.*, C5, C6, C7, or C8).

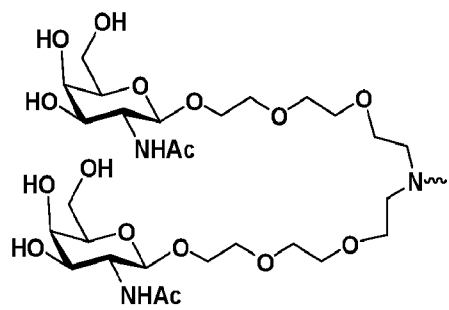
In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



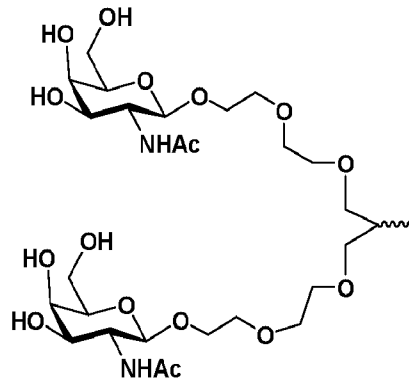
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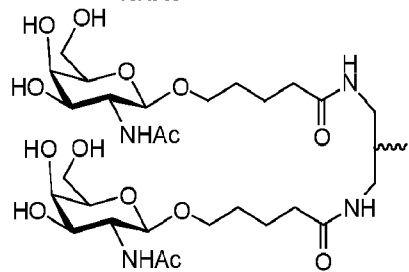
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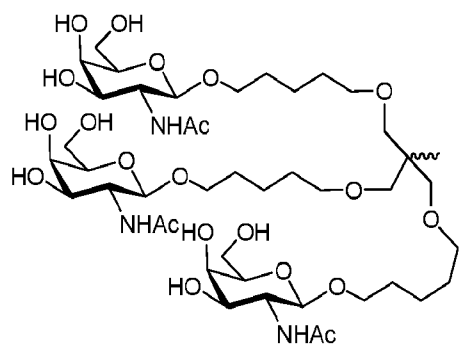
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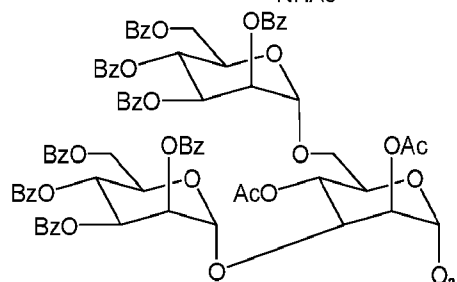
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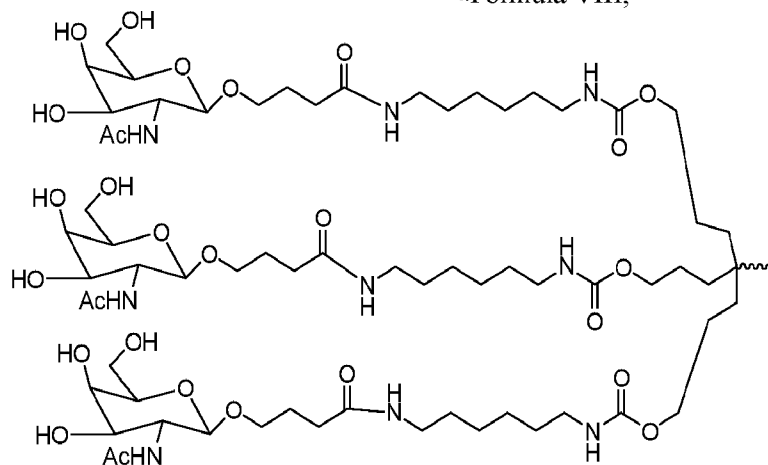
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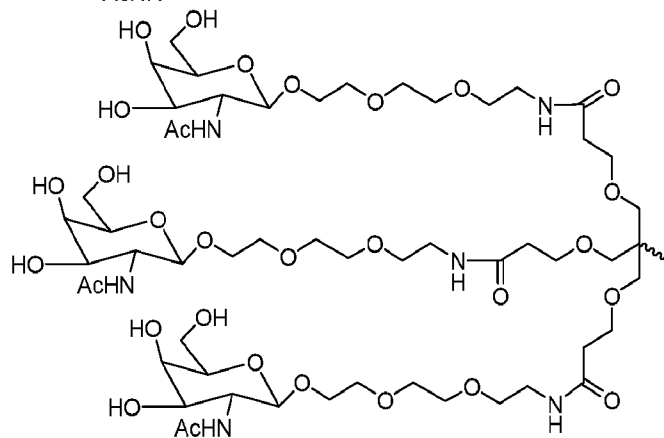
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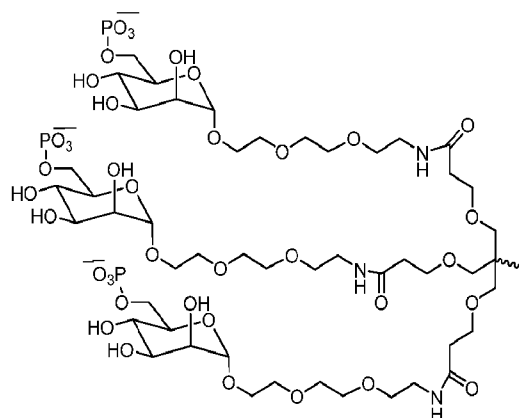
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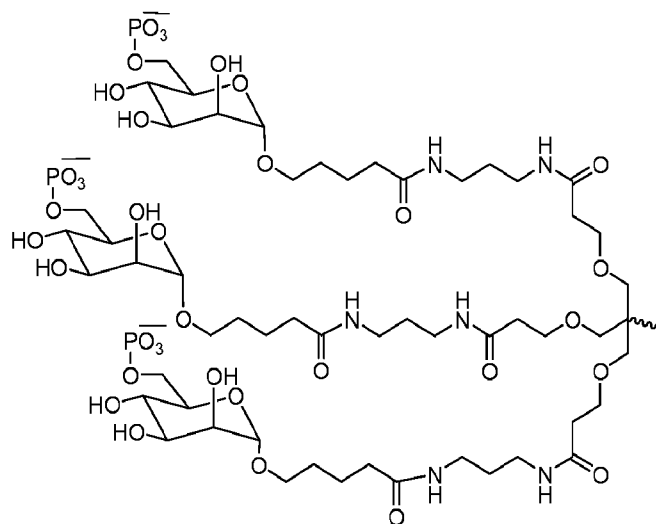
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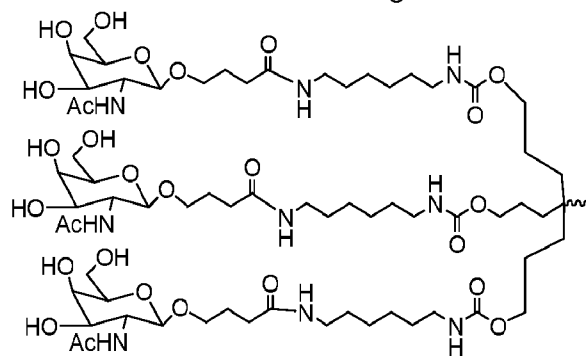
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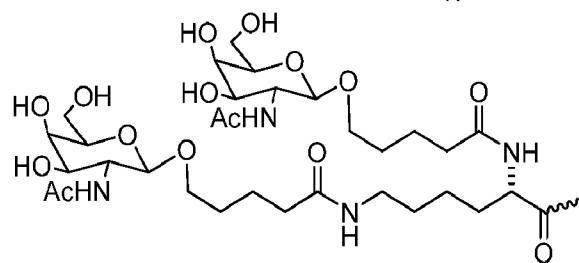
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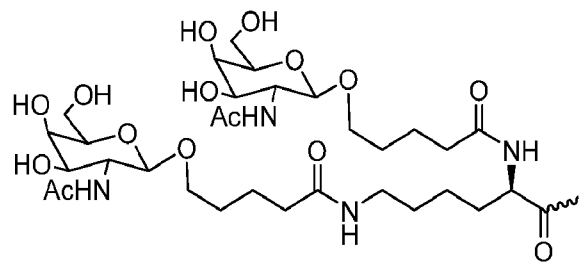
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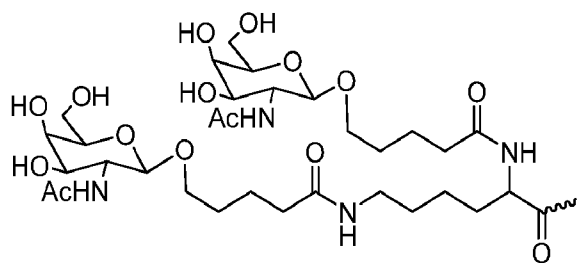
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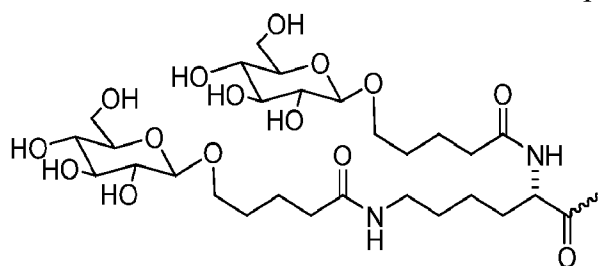
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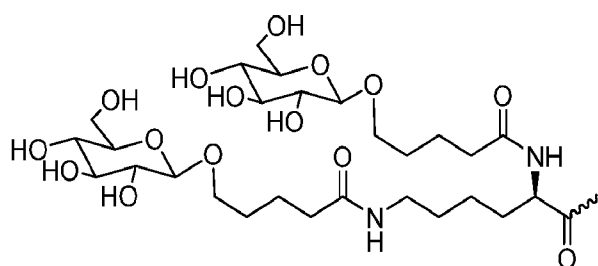
Formula XV,



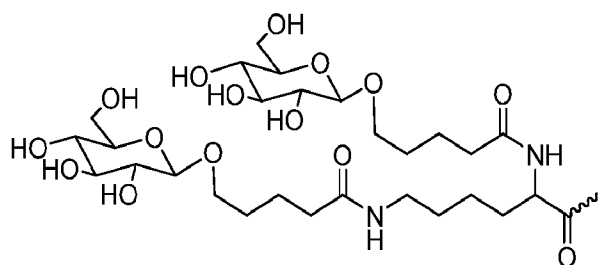
Formula XVI,



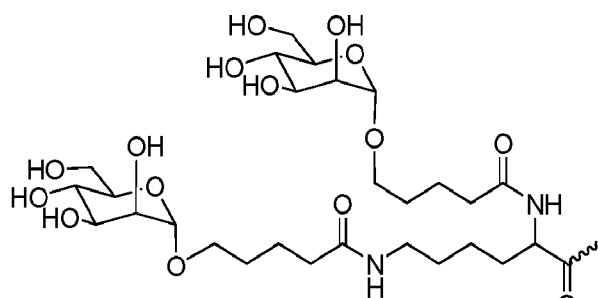
Formula XVII,



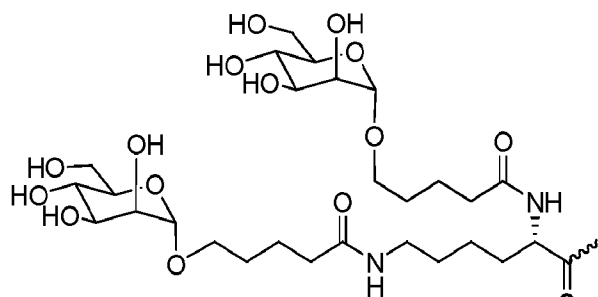
Formula XVIII,



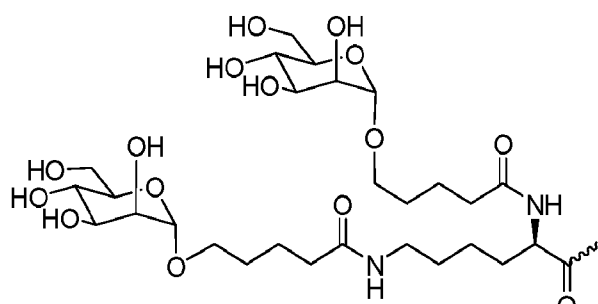
Formula XIX,



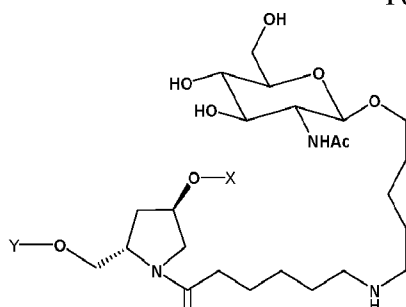
Formula XX,



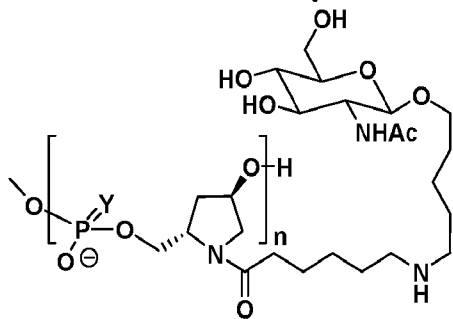
Formula XXI,



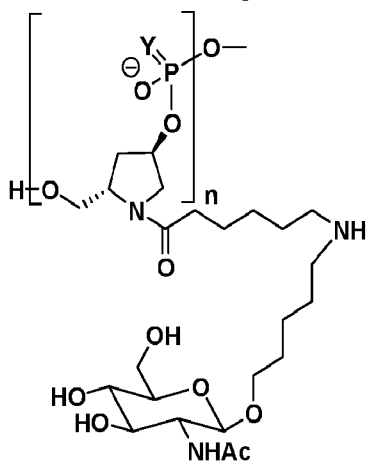
Formula XXII,



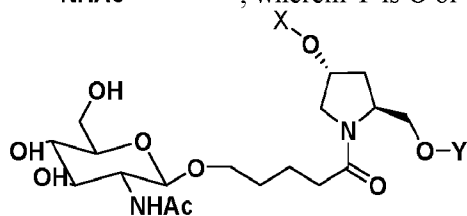
Formula XXIII;



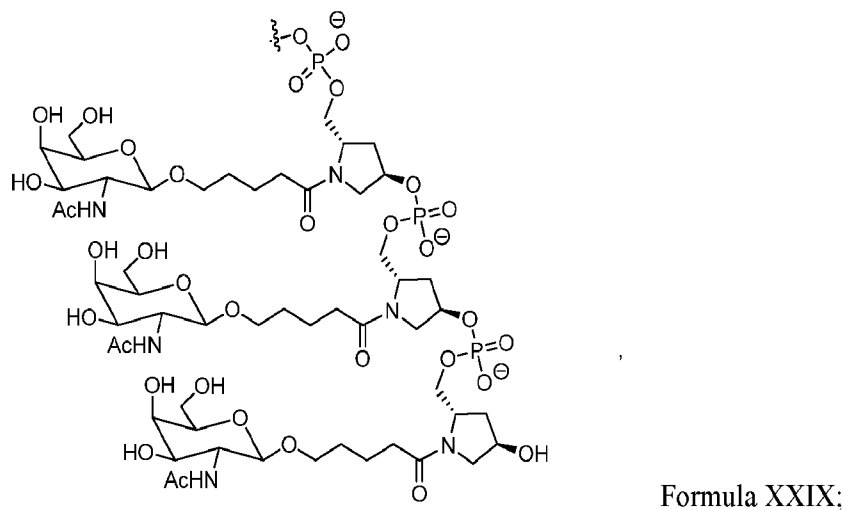
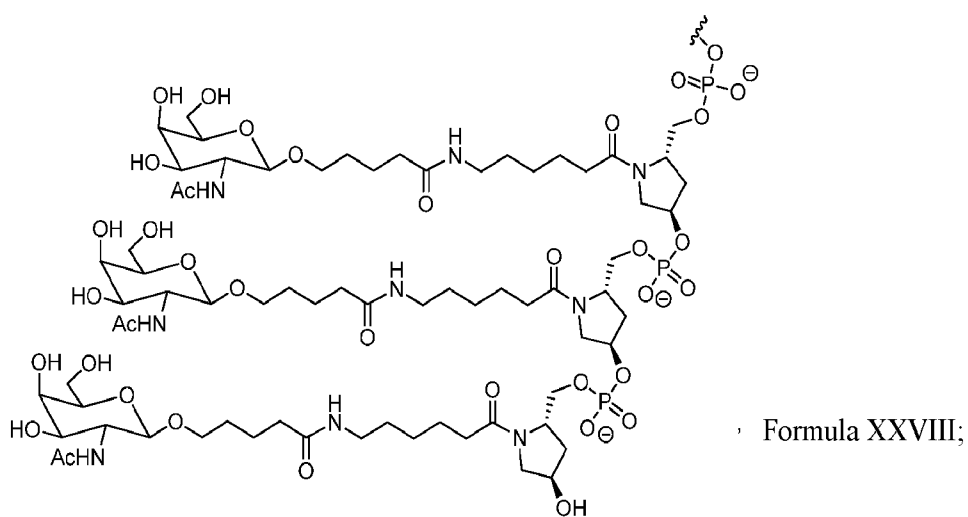
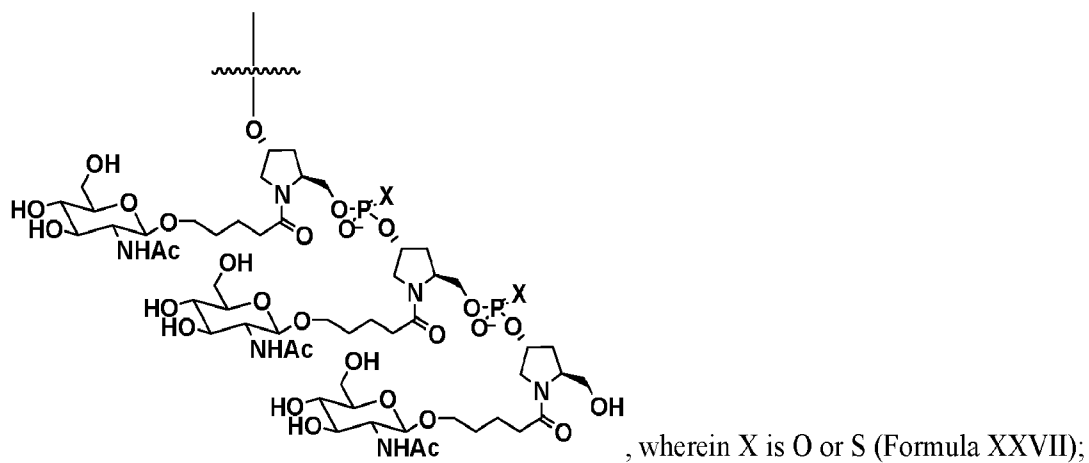
, wherein Y is O or S and n is 3 -6 (Formula XXIV);

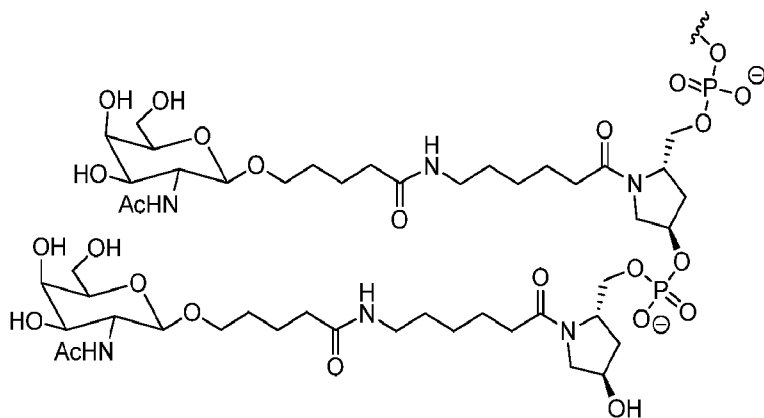


, wherein Y is O or S and n is 3-6 (Formula XXV);

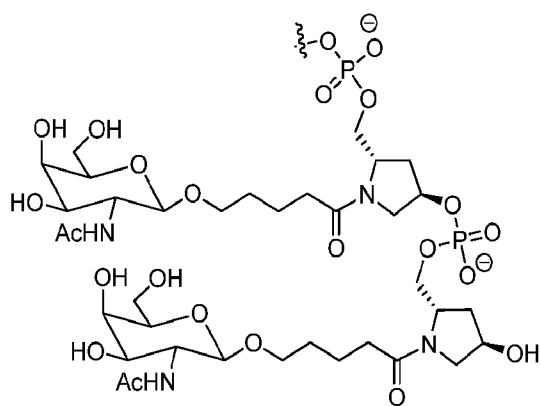


Formula XXVI;

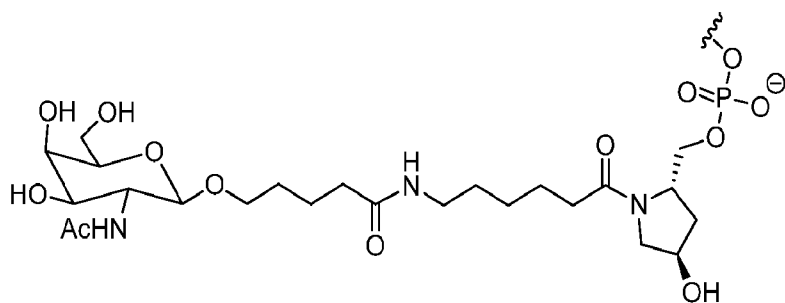
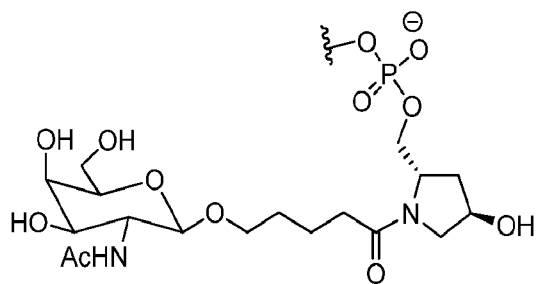




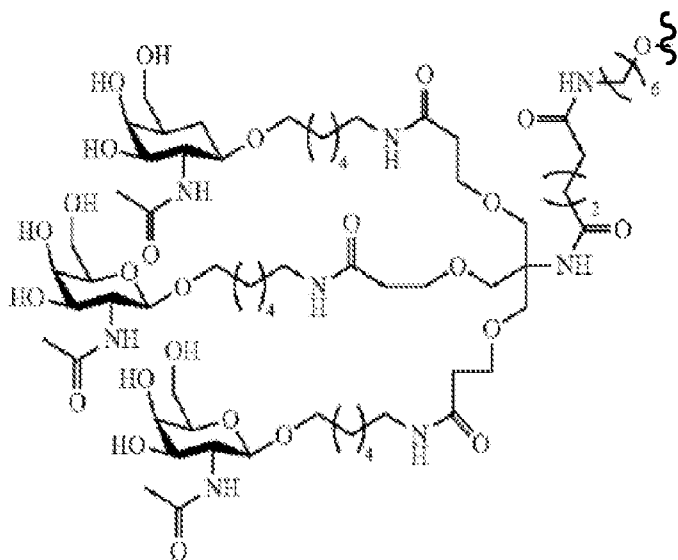
Formula XXX;



Formula XXXI;

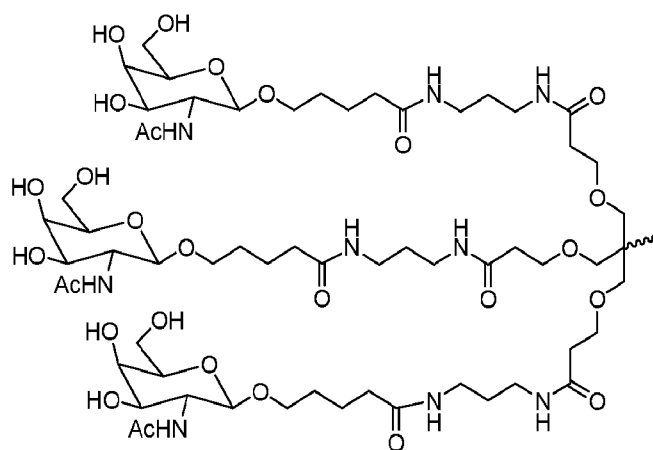
Formula XXXII;
, and

Formula XXXIII.



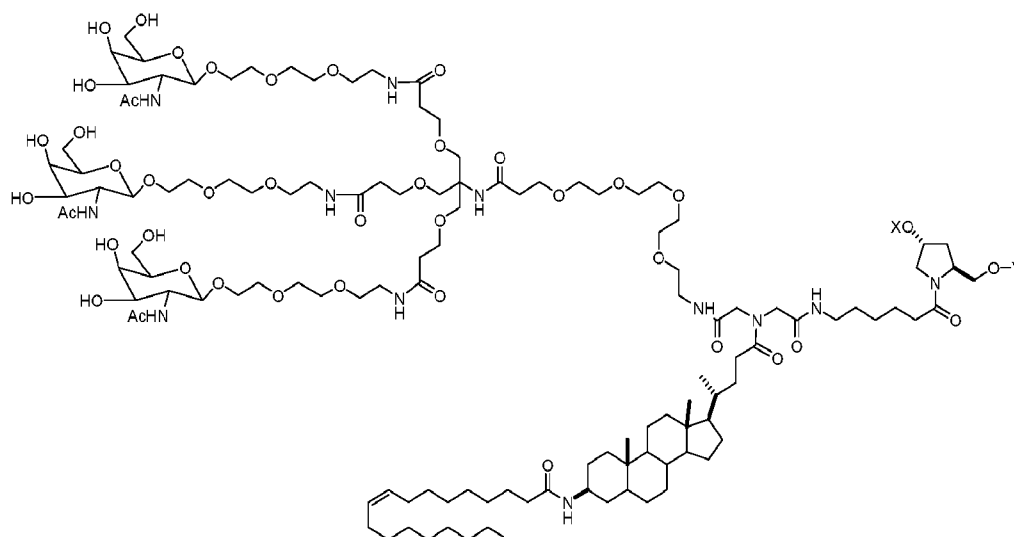
Formula XXXIV.

In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In one embodiment, the monosaccharide is an N-acetylgalactosamine, such as



Formula II.

Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



(Formula XXXV),

when one of X or Y is an oligonucleotide, the other is a hydrogen.

In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a trivalent linker.

In one embodiment, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent, *e.g.*, the 3' or 5' end of the sense strand of a dsRNA agent as described herein. In another embodiment, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) of GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker.

In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator and/or a cell permeation peptide.

Additional carbohydrate conjugates (and linkers) suitable for use in the present invention include those described in PCT Publication Nos. WO 2014/179620 and WO 2014/179627, the entire contents of each of which are incorporated herein by reference.

D. Linkers

In some embodiments, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.*, covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR₈, C(O), C(O)NH, SO, SO₂, SO₂NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclalkyl, heterocyclalkenyl, heterocyclalkynyl, aryl, heteroaryl, heterocycl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclalkyl, alkylheterocyclalkenyl, alkylheterocyclalkynyl, alkenylheterocyclalkyl, alkenylheterocyclalkenyl, alkenylheterocyclalkynyl, alkynylheterocyclalkyl, alkynylheterocyclalkenyl, alkynylheterocyclalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylheteroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO₂, N(R₈), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R₈ is hydrogen, acyl, aliphatic or substituted aliphatic. In one embodiment, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-17, or 8-16 atoms.

A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times or more, or at least about 100 times faster in a target cell or under a first reference condition (which can, *e.g.*, be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, *e.g.*, be selected to mimic or represent conditions found in the blood or serum).

Cleavable linking groups are susceptible to cleavage agents, *e.g.*, pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, *e.g.*, oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, *e.g.*, those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes

have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, *e.g.*, blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

i. Redox cleavable linking groups

In one embodiment, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents known in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under

conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

ii. Phosphate-based cleavable linking groups

In another embodiment, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -O-P(S)(Rk)-S-, -O-P(S)(Rk)-S-. Preferred embodiments are -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -S-P(S)(OH)-O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O-, -S-P(S)(H)-O-, -S-P(O)(H)-S-, -O-P(S)(H)-S-. A preferred embodiment is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

In another embodiment, a cleavable linker comprises an acid cleavable linking group. An acid cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (*e.g.*, about 6.0, 5.75, 5.5, 5.25, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula -C=NN-, C(O)O, or -OC(O). A preferred embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or *t*-butyl. These candidates can be evaluated using methods analogous to those described above.

iv. Ester-based linking groups

In another embodiment, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula -C(O)O-, or -OC(O)-.

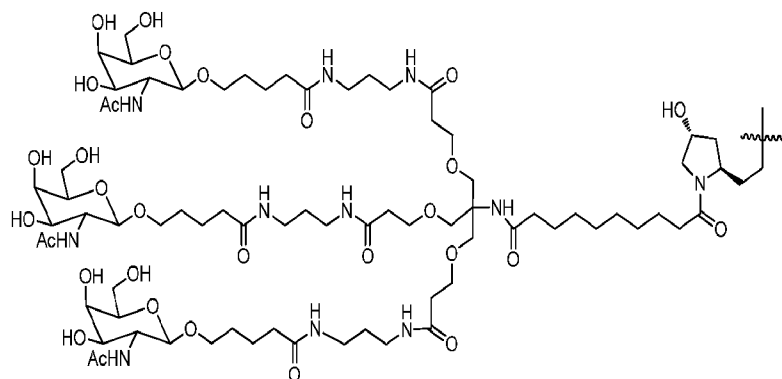
These candidates can be evaluated using methods analogous to those described above.

v. Peptide-based cleaving groups

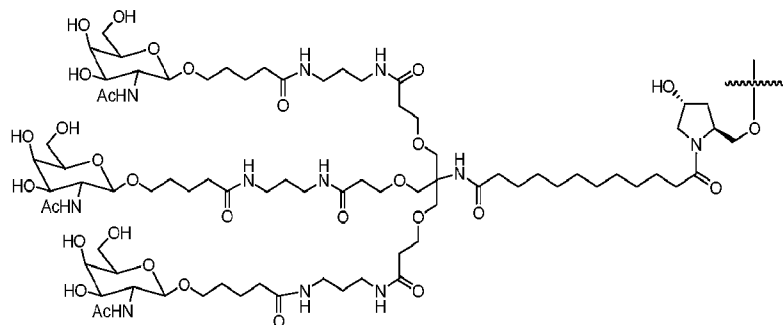
In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides *etc.*) and polypeptides. Peptide-based cleavable groups do not include the amide

group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or alkynylene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula -NHCHRAC(O)NHCHRBC(O)-, where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

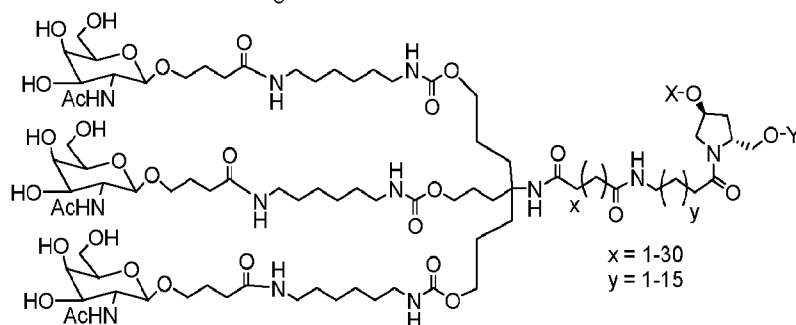
In one embodiment, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



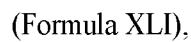
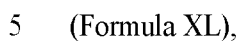
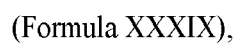
(Formula XXXVI),



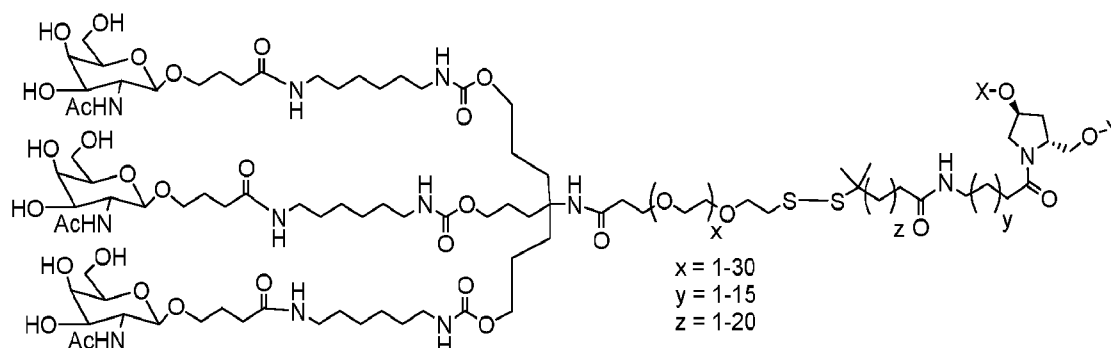
(Formula XXXVII),



(Formula XXXVIII),



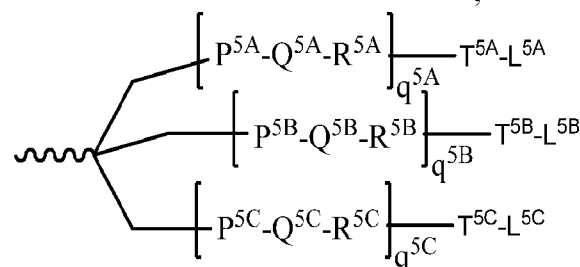
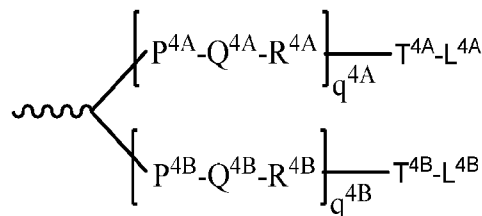
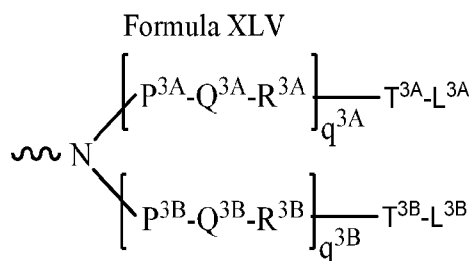
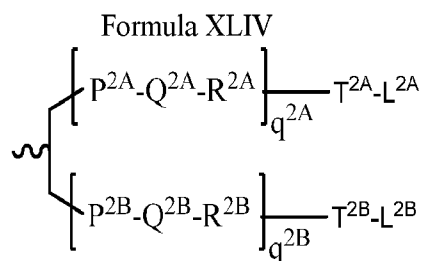
(Formula XLII), and



(Formula XLIII), when one of X or Y is an oligonucleotide, the other is a hydrogen.

In certain embodiments of the compositions and methods of the invention, a ligand is one or more
 5 GalNAc (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of Formula XLIV – XLVII:



10

Formula XLVI

Formula XLVII

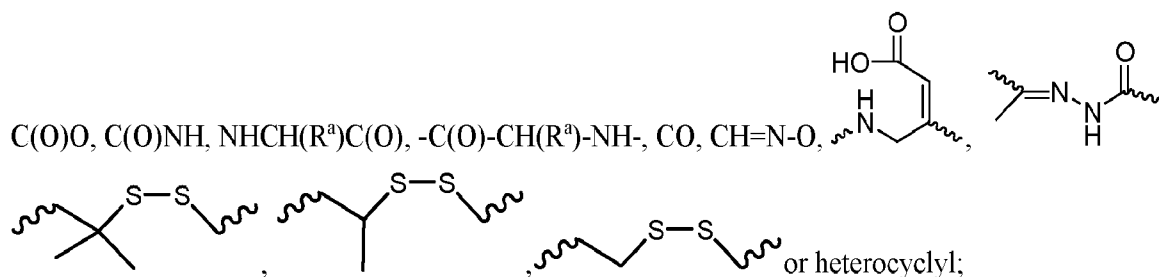
wherein:

q^{2A}, q^{2B}, q^{3A}, q^{3B}, q^{4A}, q^{4B}, q^{5A}, q^{5B} and q^{5C} represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

15 P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH₂, CH₂NH or CH₂O;

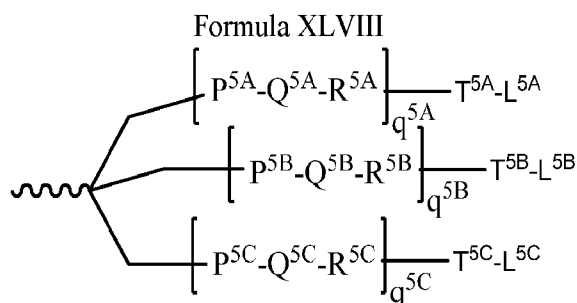
Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO₂, N(R^N), C(R')=C(R''), C≡C or C(O);

R^{2A} , R^{2B} , R^{3A} , R^{3B} , R^{4A} , R^{4B} , R^{5A} , R^{5B} , R^{5C} are each independently for each occurrence absent, NH, O, S, CH₂,



L^{2A} , L^{2B} , L^{3A} , L^{3B} , L^{4A} , L^{4B} , L^{5A} , L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each

5 occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; and R^a is H or amino acid side chain. Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula XLVIII:



10 wherein L^{5A} , L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the structures recited above as formulas II, VII, XI, X, and XIII.

Representative U.S. patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single

nucleoside within an iRNA. The present invention also includes iRNA compounds that are chimeric compounds.

“Chimeric” iRNA compounds or “chimeras,” in the context of this invention, are iRNA compounds, preferably dsRNAs, which contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.*, a nucleotide in the case of a dsRNA compound. These iRNAs typically contain at least one region wherein the RNA is modified so as to confer upon the iRNA increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the iRNA can serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of iRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter iRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxy dsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to enhance the activity, cellular distribution or cellular uptake of the iRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. *et al.*, Biochem. Biophys. Res. Comm., 2007, 365(1):54-61; Letsinger *et al.*, Proc. Natl. Acad. Sci. USA, 1989, 86:6553), cholic acid (Manoharan *et al.*, Bioorg. Med. Chem. Lett., 1994, 4:1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al.*, Ann. N.Y. Acad. Sci., 1992, 660:306; Manoharan *et al.*, Bioorg. Med. Chem. Lett., 1993, 3:2765), a thiocholesterol (Oberhauser *et al.*, Nucl. Acids Res., 1992, 20:533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, EMBO J., 1991, 10:111; Kabanov *et al.*, FEBS Lett., 1990, 259:327; Svinarchuk *et al.*, Biochimie, 1993, 75:49), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, Tetrahedron Lett., 1995, 36:3651; Shea *et al.*, Nucl. Acids Res., 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, Nucleosides & Nucleotides, 1995, 14:969), or adamantane acetic acid (Manoharan *et al.*, Tetrahedron Lett., 1995, 36:3651), a palmityl moiety (Mishra *et al.*, Biochim. Biophys. Acta, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke *et al.*, J. Pharmacol. Exp. Ther., 1996, 277:923). Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of an RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following

cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

V. Delivery of an iRNA of the Invention

The delivery of an iRNA of the invention to a cell *e.g.*, a cell within a subject, such as a human subject (*e.g.*, a subject in need thereof, such as a subject having a disorder of lipid metabolism) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an iRNA of the invention either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an iRNA, *e.g.*, a dsRNA, to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the iRNA. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an iRNA of the invention (see *e.g.*, Akhtar S. and Julian RL., (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an iRNA molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. The non-specific effects of an iRNA can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the iRNA molecule to be administered. Several studies have shown successful knockdown of gene products when an iRNA is administered locally. For example, intraocular delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, MJ. *et al.*, (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, SJ. *et al.* (2003) *Mol. Vis.* 9:210-216) were both shown to prevent neovascularization in an experimental model of age-related macular degeneration. In addition, direct intratumoral injection of a dsRNA in mice reduces tumor volume (Pille, J. *et al.* (2005) *Mol. Ther.* 11:267-274) and can prolong survival of tumor-bearing mice (Kim, WJ. *et al.*, (2006) *Mol. Ther.* 14:343-350; Li, S. *et al.*, (2007) *Mol. Ther.* 15:515-523). RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G. *et al.*, (2004) *Nucleic Acids* 32:e49; Tan, PH. *et al.* (2005) *Gene Ther.* 12:59-66; Makimura, H. *et al.* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al.* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al.* (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya, Y., *et al.* (2005) *J. Neurophysiol.* 93:594-602) and to the lungs by intranasal administration (Howard, KA. *et al.*, (2006) *Mol. Ther.* 14:476-484; Zhang, X. *et al.*, (2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V. *et al.*, (2005) *Nat. Med.* 11:50-55). For administering an iRNA systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the dsRNA by endo- and exo-

nucleases *in vivo*. Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA composition to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J. *et al.*, (2004) *Nature* 432:173-178). Conjugation of an iRNA to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, JO. *et al.*, (2006) *Nat. Biotechnol.* 24:1005-1015). In an alternative embodiment, the iRNA can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an iRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, or induced to form a vesicle or micelle (see *e.g.*, Kim SH. *et al.*, (2008) *Journal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic- iRNA complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR., *et al.* (2003) *J. Mol. Biol* 327:761-766; Verma, UN. *et al.*, (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, AS *et al.*, (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of iRNAs include DOTAP (Sorensen, DR., *et al.* (2003), *supra*; Verma, UN. *et al.*, (2003), *supra*), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS. *et al.*, (2006) *Nature* 441:111-114), cardiolipin (Chien, PY. *et al.*, (2005) *Cancer Gene Ther.* 12:321-328; Pal, A. *et al.*, (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME. *et al.*, (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA. *et al.*, (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H. *et al.*, (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an iRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of iRNAs and cyclodextrins can be found in U.S. Patent No. 7, 427, 605, which is herein incorporated by reference in its entirety.

A. Vector encoded iRNAs of the Invention

iRNA targeting the CIDEA gene can be expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A. *et al.*, *TIG.* (1996), 12:5-10; Skillern, A., *et al.*, International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, U.S. Pat. No. 6,054,299). Expression can be transient (on the order of hours to weeks) or sustained (weeks to months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-

integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, (1995) *Proc. Natl. Acad. Sci. USA* 92:1292).

The individual strand or strands of an iRNA can be transcribed from a promoter on an expression vector. Where two separate strands are to be expressed to generate, for example, a dsRNA, two separate
5 expression vectors can be co-introduced (*e.g.*, by transfection or infection) into a target cell. Alternatively, each individual strand of a dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a dsRNA is expressed as inverted repeat polynucleotides joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

iRNA expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible
10 with eukaryotic cells, preferably those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of an iRNA as described herein. Eukaryotic cell expression vectors are well known in the art and are available from a number of commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired nucleic acid segment. Delivery of iRNA
15 expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to
20 lentiviral vectors, moloney murine leukemia virus, *etc.*; (c) adeno- associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.*, vaccinia virus vectors or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs
25 can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. Constructs for the recombinant expression of an iRNA will generally require regulatory elements, *e.g.*, promoters, enhancers, *etc.*, to ensure the expression of the iRNA in target cells. Other aspects to consider for vectors and constructs are known in the art.

30 **VI. Pharmaceutical Compositions of the Invention**

The present invention also includes pharmaceutical compositions and formulations which include the iRNAs of the invention. Accordingly, in one embodiment, provided herein are pharmaceutical compositions comprising a double stranded ribonucleic acid (dsRNA) agent that inhibits expression of cell death-inducing
35 DFFA-like effector b (CIDEb) in a cell, such as a liver cell, wherein the dsRNA agent comprises a sense strand and an antisense strand, wherein the sense strand comprises at least 15 contiguous nucleotides differing

by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO: 1, and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO: 2; and a pharmaceutically acceptable carrier. In some embodiments, the dsRNA agent comprises a sense strand and an antisense strand, wherein the sense strand comprises at least 15 contiguous nucleotides from the nucleotide sequence of SEQ ID NO: 1, and said antisense strand comprises at least 15 contiguous nucleotides from the nucleotide sequence of SEQ ID NO: 2.

In another embodiment, provided herein are pharmaceutical compositions comprising a dsRNA agent that inhibits expression of CIDEB in a cell, such as a liver cell, wherein the dsRNA agent comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from any one of the antisense sequences listed in Tables 3-6; and a pharmaceutically acceptable carrier. In some embodiments, the dsRNA agent comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides from any one of the antisense sequences listed in Tables 3-6.

The pharmaceutical compositions containing the iRNA of the invention are useful for treating a disease or disorder associated with the expression or activity of a CIDEB gene, *e.g.*, a chronic inflammatory disease.

Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery, *e.g.*, by intravenous (IV), intramuscular (IM) or for subcutaneous delivery. Another example is compositions that are formulated for direct delivery into the liver, *e.g.*, by infusion into the liver, such as by continuous pump infusion. The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of a CIDEB gene. In general, a suitable dose of an iRNA of the invention will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. Typically, a suitable dose of an iRNA of the invention will be in the range of about 0.1 mg/kg to about 5.0 mg/kg, preferably about 0.3 mg/kg and about 3.0 mg/kg. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as every other day to once a year. In certain embodiments, the iRNA is administered about once per week, once every 7-10 days, once every 2 weeks, once every 3 weeks, once every 4 weeks, once every 5 weeks, once every 6 weeks, once every 7 weeks, once every 8 weeks, once every 9 weeks, once every 10 weeks, once every 11 weeks, once every 12 weeks, once per month, once every 2 months, once every 3 months (once per quarter), once every 4 months, once every 5 months, or once every 6 months.

After an initial treatment regimen, the treatments can be administered on a less frequent basis.

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous

treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and *in vivo* half-lives for the individual iRNAs encompassed by the invention can be made using conventional methodologies or on the basis of *in vivo* testing using an

appropriate animal model, as described elsewhere herein.

Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as a CIDEB-associated disease, disorder, or condition that would benefit from reduction in the expression of CIDEB. Such models can be used for *in vivo* testing of iRNA, as well as for determining a therapeutically effective dose. Such models can be used for *in vivo* testing of iRNA, as well as for determining a therapeutically effective dose. Suitable mouse models are known in the art and include, for example, mice and rats fed a high fat diet (HFD; also referred to as a Western diet), a methionine-choline deficient (MCD) diet, or a high-fat (15%), high-cholesterol (1%) diet (HFHC), an obese (ob/ob) mouse containing a mutation in the obese (ob) gene (Wiegman *et al.*, (2003) *Diabetes*, 52:1081-1089); a mouse containing homozygous knock-out of an LDL receptor (LDLR ^{-/-} mouse; Ishibashi *et al.*, (1993) *J Clin Invest* 92(2):883-893); diet-induced atherosclerosis mouse model (Ishida *et al.*, (1991) *J. Lipid. Res.*, 32:559-568); heterozygous lipoprotein lipase knockout mouse model (Weistock *et al.*, (1995) *J. Clin. Invest.* 96(6):2555-2568); mice and rats fed a choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD) (Matsumoto *et al.* (2013) *Int. J. Exp. Path.* 94:93-103); mice and rats fed a high-trans-fat, cholesterol diet (HTF-C) (Clapper *et al.* (2013) *Am. J. Physiol. Gastrointest. Liver Physiol.* 305:G483-G495); mice and rats fed a high-fat, high-cholesterol, bile salt diet (HF/HC/BS) (Matsuzawa *et al.* (2007) *Hepatology* 46:1392-1403); and mice and rats fed a high-fat diet + fructose (30%) water (Softic *et al.* (2018) *J. Clin. Invest.* 128(1)-85-96).

The pharmaceutical compositions of the present invention can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be topical (*e.g.*, by a transdermal patch), pulmonary, *e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; subdermal, *e.g.*, via an implanted device; or intracranial, *e.g.*, by intraparenchymal, intrathecal or intraventricular, administration.

The iRNA can be delivered in a manner to target a particular cell or tissue, such as the liver (*e.g.*, the hepatocytes of the liver).

In some embodiments, the pharmaceutical compositions of the invention are suitable for intramuscular administration to a subject. In other embodiments, the pharmaceutical compositions of the invention are suitable for intravenous administration to a subject. In some embodiments of the invention, the pharmaceutical compositions of the invention are suitable for subcutaneous administration to a subject, *e.g.*, using a 29g or 30g needle.

The pharmaceutical compositions of the invention may include an RNAi agent of the invention in an unbuffered solution, such as saline or water, or in a buffer solution, such as a buffer solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

In one embodiment, the pharmaceutical compositions of the invention, *e.g.*, such as the compositions suitable for subcutaneous administration, comprise an RNAi agent of the invention in phosphate buffered saline (PBS). Suitable concentrations of PBS include, for example, 1mM, 1.5 mM, 2 mM, 2.5 mM, 3 mM, 3.5 mM, 4 mM, 4.5 mM, 5 mM, 6.5 mM, 7 mM, 7.5 mM, 9 mM, 8.5 mM, 9 mM, 9.5 mM, or about 10 mM PBS. In one embodiment of the invention, a pharmaceutical composition of the invention comprises an RNAi agent of the invention dissolved in a solution of about 5 mM PBS (*e.g.*, 0.64 mM NaH_2PO_4 , 4.36 mM Na_2HPO_4 , 85 mM NaCl). Values intermediate to the above recited ranges and values are also intended to be part of this invention. In addition, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included.

The pH of the pharmaceutical compositions of the invention may be between about 5.0 to about 8.0, about 5.5 to about 8.0, about 6.0 to about 8.0, about 6.5 to about 8.0, about 7.0 to about 8.0, about 5.0 to about 7.5, about 5.5 to about 7.5, about 6.0 to about 7.5, about 6.5 to about 7.5, about 5.0 to about 7.2, about 5.25 to about 7.2, about 5.5 to about 7.2, about 5.75 to about 7.2, about 6.0 to about 7.2, about 6.5 to about 7.2, or about 6.8 to about 7.2. Ranges and values intermediate to the above recited ranges and values are also intended to be part of this invention.

The osmolality of the pharmaceutical compositions of the invention may be suitable for subcutaneous administration, such as no more than about 400 mOsm/kg, *e.g.*, between 50 and 400 mOsm/kg, between 75 and 400 mOsm/kg, between 100 and 400 mOsm/kg, between 125 and 400 mOsm/kg, between 150 and 400 mOsm/kg, between 175 and 400 mOsm/kg, between 200 and 400 mOsm/kg, between 250 and 400 mOsm/kg, between 300 and 400 mOsm/kg, between 50 and 375 mOsm/kg, between 75 and 375 mOsm/kg, between 100 and 375 mOsm/kg, between 125 and 375 mOsm/kg, between 150 and 375 mOsm/kg, between 175 and 375 mOsm/kg, between 200 and 375 mOsm/kg, between 250 and 375 mOsm/kg, between 300 and 375 mOsm/kg, between 50 and 350 mOsm/kg, between 75 and 350 mOsm/kg, between 100 and 350 mOsm/kg, between 125 and 350 mOsm/kg, between 150 and 350 mOsm/kg, between 175 and 350 mOsm/kg, between 200 and 350 mOsm/kg, between 250 and 350 mOsm/kg, between 50 and 325 mOsm/kg, between 75 and 325 mOsm/kg, between 100 and 325 mOsm/kg, between 125 and 325 mOsm/kg, between 150 and 325 mOsm/kg, between 175 and 325 mOsm/kg, between 200 and 325 mOsm/kg, between 250 and 325 mOsm/kg, between 300 and 325 mOsm/kg, between 300 and 350 mOsm/kg, between 50 and 300 mOsm/kg, between 75 and 300 mOsm/kg, between 100 and 300 mOsm/kg, between 125 and 300 mOsm/kg, between 150 and 300 mOsm/kg, between 175 and 300 mOsm/kg, between 200 and 300 mOsm/kg, between 250 and 300, between 50 and 250 mOsm/kg, between 75 and 250 mOsm/kg, between 100 and 250 mOsm/kg, between 125 and 250 mOsm/kg, between 150 and 250 mOsm/kg, between 175 and 350 mOsm/kg, between 200 and 250 mOsm/kg, *e.g.*, about 50, 55, 60, 65, 70, 75, 80, 85, 90, 95,

100, 105, 110, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 295, 300, 305, 310, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, or about 400 mOsm/kg. Ranges and values intermediate to the above recited ranges and values are also intended to be part of this invention.

5 The pharmaceutical compositions of the invention comprising the RNAi agents of the invention, may be present in a vial that contains about 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or about 2.0 mL of the pharmaceutical composition. The concentration of the RNAi agents in the pharmaceutical compositions of the invention may be about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 130, 125, 130, 135, 140, 145, 150, 175, 180, 185, 190, 195, 200, 205, 210, 215, 230, 225, 230, 235, 240, 245, 10 250, 275, 280, 285, 290, 295, 300, 305, 310, 315, 330, 325, 330, 335, 340, 345, 350, 375, 380, 385, 390, 395, 400, 405, 410, 415, 430, 425, 430, 435, 440, 445, 450, 475, 480, 485, 490, 495, or about 500 mg/mL. In one embodiment, the concentration of the RNAi agents in the pharmaceutical compositions of the invention is about 100 mg/mL. Values intermediate to the above recited ranges and values are also intended to be part of this invention.

15 The pharmaceutical compositions of the invention may comprise a dsRNA agent of the invention in a free acid form. In other embodiments of the invention, the pharmaceutical compositions of the invention may comprise a dsRNA agent of the invention in a salt form, such as a sodium salt form. In certain embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for substantially all of the phosphodiester and/or phosphorothioate groups present in the agent. Agents in which 20 substantially all of the phosphodiester and/or phosphorothioate linkages have a sodium counterion include not more than 5, 4, 3, 2, or 1 phosphodiester and/or phosphorothioate linkages without a sodium counterion. In some embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for all of the phosphodiester and/or phosphorothioate groups present in the agent.

Pharmaceutical compositions and formulations for topical administration can include transdermal 25 patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable. Coated condoms, gloves and the like can also be useful. Suitable topical formulations include those in which the iRNAs featured in the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Suitable lipids and liposomes include 30 neutral (*e.g.*, dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline) negative (*e.g.*, dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.*, dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). iRNAs featured in the invention can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, iRNAs can be complexed to lipids, in particular to cationic lipids. Suitable fatty 35 acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic

acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C₁₋₂₀ alkyl ester (e.g., isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in U.S. Patent No.

6,747,014, which is incorporated herein by reference.

A. *iRNA Formulations Comprising Membranous Molecular Assemblies*

An iRNA for use in the compositions and methods of the invention can be formulated for delivery in a membranous molecular assembly, e.g., a liposome or a micelle. There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present invention, the term “liposome” means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

Liposomes include unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition (e.g., iRNA) to be delivered. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the iRNA composition, although in some examples, it may. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages *in vivo*.

In order to traverse intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes and as the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act.

Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into the skin. Compounds including analgesics, antibodies, hormones and high-molecular

weight DNAs have been administered to the skin. The majority of applications resulted in the targeting of the upper epidermis.

A liposome containing an iRNA agent can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical micelle concentration and may be nonionic. Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The iRNA agent preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the iRNA agent and condense around the iRNA agent to form a liposome. After condensation, the detergent is removed, e.g., by dialysis, to yield a liposomal preparation of iRNA agent.

If necessary a carrier compound that assists in condensation can be added during the condensation reaction, e.g., by controlled addition. For example, the carrier compound can be a polymer other than a nucleic acid (e.g., spermine or spermidine). pH can also be adjusted to favor condensation.

Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as structural components of the delivery vehicle, are further described in, e.g., WO 96/37194, the entire contents of which are incorporated herein by reference. Liposome formation can also include one or more aspects of exemplary methods described in Felgner, P. L. et al., Proc. Natl. Acad. Sci., USA 8:7413-7417, 1987; US Patent No. 4,897,355; US Patent No. 5,171,678; Bangham, et al. M. Mol. Biol. 23:238, 1965; Olson, et al. Biochim. Biophys. Acta 557:9, 1979; Szoka, et al. Proc. Natl. Acad. Sci. 75: 4194, 1978; Mayhew, et al. Biochim. Biophys. Acta 775:169, 1984; Kim, et al. Biochim. Biophys. Acta 728:339, 1983; and Fukunaga, et al. Endocrinol. 115:757, 1984. Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, e.g., Mayer, et al. Biochim. Biophys. Acta 858:161, 1986). Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew, et al. Biochim. Biophys. Acta 775:169, 1984). These methods are readily adapted to packaging iRNA agent preparations into liposomes.

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged DNA molecules to form a stable complex. The positively charged DNA/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al., Biochem. Biophys. Res. Commun., 1987, 147, 980-985).

Liposomes which are pH-sensitive or negatively-charged, entrap DNA rather than complex with it. Since both the DNA and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some DNA is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell

monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou *et al.*, *Journal of Controlled Release*, 1992, 19, 269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

Examples of other methods to introduce liposomes into cells *in vitro* and *in vivo* include US Patent Nos. 5,283,185 and 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Felgner, J. Biol. Chem. 269:2550, 1994; Nabel, Proc. Natl. Acad. Sci. 90:11307, 1993; Nabel, Human Gene Ther. 3:649, 1992; Gershon, Biochem. 32:7143, 1993; and Strauss EMBO J. 11:417, 1992.

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporin-A into different layers of the skin (Hu *et al.* S.T.P. Pharma. Sci., 1994, 4, 6, 466).

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen *et al.*, *FEBS Letters*, 1987, 223, 42; Wu *et al.*, *Cancer Research*, 1993, 53, 3765).

Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos *et al.* (*Ann. N.Y. Acad. Sci.*, 1987, 507, 64) reported the ability of monosialoganglioside G_{M1}, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, 1988, 85, 6949). U.S. Pat.

No. 4,837,028 and WO 88/04924, both to Allen *et al.*, disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb *et al.*) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim *et al.*).

5 In some embodiments, cationic liposomes are used. Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-cationic liposomes, although not able to fuse as efficiently with the plasma membrane, are taken up by macrophages *in vivo* and can be used to deliver iRNA agents to macrophages.

Further advantages of liposomes include: liposomes obtained from natural phospholipids are
10 biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated iRNAs in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and Banker (Eds.), 1988, volume 1, p.245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size, and the aqueous volume of the liposomes.

15 A positively charged synthetic cationic lipid, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells, resulting in delivery of iRNA agent (see, e.g., Felgner, P. L. et al., Proc. Natl. Acad. Sci., USA 8:7413-7417, 1987 and US Patent No.4,897,355 for a
20 description of DOTMA and its use with DNA).

A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. Lipofectin™ (Bethesda Research Laboratories, Gaithersburg, Md.) is an effective agent for the delivery of highly anionic nucleic acids into living tissue culture cells that comprise positively charged DOTMA liposomes which interact
25 spontaneously with negatively charged polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive. Positively charged complexes prepared in this way spontaneously attach to negatively charged cell surfaces, fuse with the plasma membrane, and efficiently deliver functional nucleic acids into, for example, tissue culture cells. Another commercially available cationic lipid, 1,2-bis(oleoyloxy)-3,3-(trimethylammonia)propane
30 ("DOTAP") (Boehringer Mannheim, Indianapolis, Indiana) differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages.

Other reported cationic lipid compounds include those that have been conjugated to a variety of moieties including, for example, carboxyspermine which has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine dioctaoyleamide ("DOGS")

(Transfectam™, Promega, Madison, Wisconsin) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-amide ("DPPEs") (see, e.g., US Patent No.5,171,678).

Another cationic lipid conjugate includes derivatization of the lipid with cholesterol ("DC- Chol") which has been formulated into liposomes in combination with DOPE (See, Gao, X. and Huang, L., Biochim. Biophys. Res. Commun.179:280, 1991). Lipopolylysine, made by conjugating polylysine to DOPE, has been reported to be effective for transfection in the presence of serum (Zhou, X. et al., Biochim. Biophys. Acta 1065:8, 1991). For certain cell lines, these liposomes containing conjugated cationic lipids, are said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing compositions. Other commercially available cationic lipid products include DMRIE and DMRIE-HP (Vical, La Jolla, California) and Lipofectamine (DOSPA) (Life Technology, Inc., Gaithersburg, Maryland). Other cationic lipids suitable for the delivery of oligonucleotides are described in WO 98/39359 and WO 96/37194.

Liposomal formulations are particularly suited for topical administration; liposomes present several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer iRNA agent into the skin. In some implementations, liposomes are used for delivering iRNA agent to epidermal cells and also to enhance the penetration of iRNA agent into dermal tissues, e.g., into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, e.g., Weiner et al., Journal of Drug Targeting, 1992, vol.2,405-410 and du Plessis et al., Antiviral Research, 18, 1992, 259-265; Mannino, R. J. and Fould-Fogerite, S., Biotechniques 6:682-690, 1988; Itani, T. et al. Gene 56:267-276, 1987; Nicolau, C. et al. Meth. Enz.149:157-176, 1987; Straubinger, R. M. and Papahadjopoulos, D. Meth. Enz.101:512-527, 1983; Wang, C. Y. and Huang, L., Proc. Natl. Acad. Sci. USA 84:7851-7855, 1987).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/ cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver a drug into the dermis of mouse skin. Such formulations with iRNA agent are useful for treating a dermatological disorder.

Liposomes that include iRNA can be made highly deformable. Such deformability can enable the liposomes to penetrate through pore that are smaller than the average radius of the liposome. For example, transfersomes are a type of deformable liposomes. Transfersomes can be made by adding surface edge activators, usually surfactants, to a standard liposomal composition. Transfersomes that include iRNAs can be delivered, for example, subcutaneously by infection in order to deliver iRNAs to keratinocytes in

the skin. In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. In addition, due to the lipid properties, these transfersomes can be self- optimizing (adaptive to the shape of pores, e.g., in the skin), self-repairing, and can frequently reach their targets without fragmenting, and often self-loading.

Other formulations amenable to the present invention are described in WO 2008/042973.

Transfersomes are yet another type of liposomes and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes may be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, e.g., they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general, their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

The iRNA for use in the methods of the invention can also be provided as micellar formulations. "Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

A mixed micellar formulation suitable for delivery through transdermal membranes may be prepared by mixing an aqueous solution of iRNA, an alkali metal C₈ to C₂₂ alkyl sulphate, and a micelle forming compounds. Exemplary micelle forming compounds include lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof. The micelle forming compounds may be added at the same time or after addition of the alkali metal alkyl sulphate. Mixed micelles will form with substantially any kind of mixing of the ingredients but vigorous mixing in order to provide smaller size micelles.

In one method a first micellar composition is prepared which contains the RNAi and at least the alkali metal alkyl sulphate. The first micellar composition is then mixed with at least three micelle forming compounds to form a mixed micellar composition. In another method, the micellar composition is prepared by mixing the RNAi, the alkali metal alkyl sulphate and at least one of the micelle forming compounds, followed by addition of the remaining micelle forming compounds, with vigorous mixing.

Phenol or m-cresol may be added to the mixed micellar composition to stabilize the formulation and protect against bacterial growth. Alternatively, phenol or m-cresol may be added with the micelle forming ingredients. An isotonic agent such as glycerin may also be added after formation of the mixed micellar composition.

For delivery of the micellar formulation as a spray, the formulation can be put into an aerosol dispenser and the dispenser is charged with a propellant. The propellant, which is under pressure, is in

liquid form in the dispenser. The ratios of the ingredients are adjusted so that the aqueous and propellant phases become one, i.e., there is one phase. If there are two phases, it is necessary to shake the dispenser prior to dispensing a portion of the contents, e.g., through a metered valve. The dispensed dose of pharmaceutical agent is propelled from the metered valve in a fine spray.

Propellants may include hydrogen-containing chlorofluorocarbons, hydrogen-containing fluorocarbons, dimethyl ether and diethyl ether. In certain embodiments, HFA 134a (1,1,1,2-tetrafluoroethane) may be used.

The specific concentrations of the essential ingredients can be determined by relatively straightforward experimentation. For absorption through the oral cavities, it is often desirable to increase, e.g., at least double or triple, the dosage for through injection or administration through the gastrointestinal tract.

B. Lipid particles

iRNAs, e.g., dsRNA agents of in the invention may be fully encapsulated in a lipid formulation, e.g., an LNP, or other nucleic acid-lipid particle.

As used herein, the term “LNP” refers to a stable nucleic acid-lipid particle. LNPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (e.g., a PEG-lipid conjugate). LNPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (e.g., sites physically separated from the administration site). As used herein, the term “SPLP” refers to a nucleic acid-lipid particle comprising plasmid DNA encapsulated within a lipid vesicle. LNPs include “pSPLP,” which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO 00/03683. The particles of the present invention typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid-lipid particles of the present invention are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, e.g., U.S. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; and PCT Publication No. WO 96/40964.

In certain embodiments, the lipid to drug ratio (mass/mass ratio) (e.g., lipid to dsRNA ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. Ranges intermediate to the above recited ranges are also contemplated to be part of the invention.

The cationic lipid may be, for example, N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-

trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3- dioleoyloxy)propylamine (DODMA), 1,2-DiLinoleoyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleoyloxy-3-(dimethylamino)acetoxyp propane (DLin-DAC), 1,2-Dilinoleoyloxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleoyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleoyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleoyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleoxylo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyldidodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid may comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

In certain embodiments, the compound 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane can be used to prepare lipid-siRNA nanoparticles. Synthesis of 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane is described in United States provisional patent application number 61/107,998 filed on October 23, 2008, which is herein incorporated by reference.

In certain embodiments, the lipid-siRNA particle includes 40% 2, 2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane: 10% DSPC: 40% Cholesterol: 10% PEG-C-DOMG (mole percent) with a particle size of 63.0 ± 20 nm and a 0.027 siRNA/Lipid Ratio.

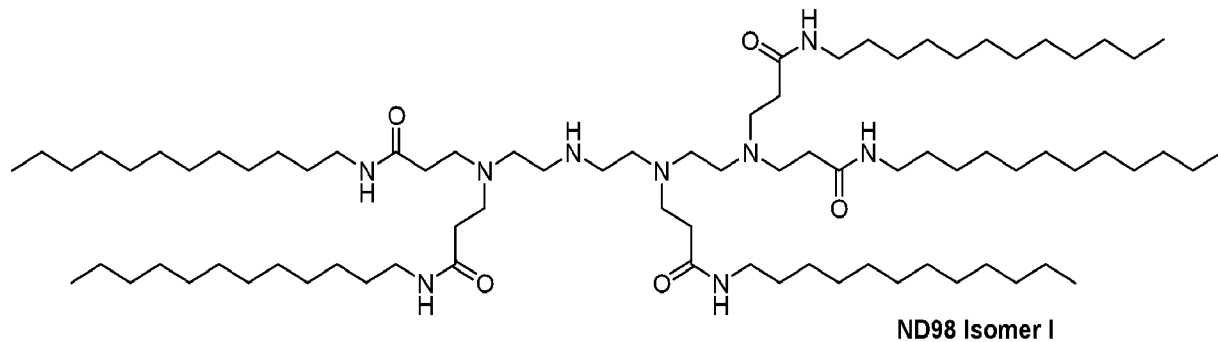
The non-cationic lipid may be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyl-oleoylphosphatidylcholine (POPC), palmitoyl-oleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1- carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1 -trans PE, 1 -stearoyl-2-oleoyl- phosphatidylethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid may be from about 5 mol % to about 90 mol %, about 10 mol %, or about 58 mol % if cholesterol is included, of the total lipid present in the particle.

The conjugated lipid that inhibits aggregation of particles may be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkylxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate may be, for example, a PEG-dilauryloxypropyl (C₁₂), a PEG-dimyristyloxypropyl (C₁₄), a PEG-dipalmitoyloxypropyl (C₁₆), or a PEG-distearoyloxypropyl (C₁₈). The conjugated lipid that prevents aggregation of particles may be from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle.

In some embodiments, the nucleic acid-lipid particle further includes cholesterol at, *e.g.*, about 10 mol % to about 60 mol % or about 48 mol % of the total lipid present in the particle.

LNP01

In certain embodiments, the lipidoid ND98·4HCl (MW 1487) (see U.S. Patent Application No. 12/056,230, filed 3/26/2008, which is herein incorporated by reference), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid-dsRNA nanoparticles (*e.g.*, LNP01 particles). Stock solutions of each in ethanol can be prepared as follows: ND98, 133 mg/ml; Cholesterol, 25 mg/ml, PEG-Ceramide C16, 100 mg/ml. The ND98, Cholesterol, and PEG-Ceramide C16 stock solutions can then be combined in a, *e.g.*, 42:48:10 molar ratio. The combined lipid solution can be mixed with aqueous dsRNA (*e.g.*, in sodium acetate pH 5) such that the final ethanol concentration is about 35-45% and the final sodium acetate concentration is about 100-300 mM. Lipid-dsRNA nanoparticles typically form spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture can be extruded through a polycarbonate membrane (*e.g.*, 100 nm cut-off) using, for example, a thermobarrel extruder, such as Lipex Extruder (Northern Lipids, Inc). In some cases, the extrusion step can be omitted. Ethanol removal and simultaneous buffer exchange can be accomplished by, for example, dialysis or tangential flow filtration. Buffer can be exchanged with, for example, phosphate buffered saline (PBS) at about pH 7, *e.g.*, about pH 6.9, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, or about pH 7.4.



Formula I

LNP01 formulations are described, *e.g.*, in International Application Publication No. WO 2008/042973, which is hereby incorporated by reference.

Additional exemplary lipid-dsRNA formulations are provided in the following Table 1.

Table 1: Exemplary lipid formulations

	Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio
SNALP	1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1
S-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:siRNA ~ 7:1
LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 6:1
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP12	1,1'-(2-(4-(2-(2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyldidodecan-2-ol (C12-200)	C12-200/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP13	XTC	XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 33:1
LNP14	MC3	MC3/DSPC/Chol/PEG-DMG 40/15/40/5 Lipid:siRNA: 11:1
LNP15	MC3	MC3/DSPC/Chol/PEG-DSG/GalNAc-PEG-DSG 50/10/35/4.5/0.5 Lipid:siRNA: 11:1

LNP16	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP17	MC3	MC3/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP18	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 12:1
LNP19	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/35/5 Lipid:siRNA: 8:1
LNP20	MC3	MC3/DSPC/Chol/PEG-DPG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP21	C12-200	C12-200/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP22	XTC	XTC/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1

DSPC: distearoylphosphatidylcholine

DPPC: dipalmitoylphosphatidylcholine

PEG-DMG: PEG-didimyristoyl glycerol (C14-PEG, or PEG-C14) (PEG with avg mol wt of 2000)

5 PEG-DSG: PEG-distyryl glycerol (C18-PEG, or PEG-C18) (PEG with avg mol wt of 2000)

PEG-cDMA: PEG-carbamoyl-1,2-dimyristyloxypropylamine (PEG with avg mol wt of 2000)

SNALP (1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO2009/127060, filed April 15, 2009, which is hereby
10 incorporated by reference.

XTC comprising formulations are described, e.g., in U.S. Provisional Serial No. 61/148,366, filed January 29, 2009; U.S. Provisional Serial No. 61/156,851, filed March 2, 2009; U.S. Provisional Serial No. 61/185,712, filed June 10, 2009; U.S. Provisional Serial No. 61/228,373, filed July 24, 2009; U.S. Provisional Serial No. 61/239,686, filed September 3, 2009, and International Application No.
15 PCT/US2010/022614, filed January 29, 2010, which are hereby incorporated by reference.

MC3 comprising formulations are described, e.g., in U.S. Provisional Serial No. 61/244,834, filed September 22, 2009, U.S. Provisional Serial No. 61/185,800, filed June 10, 2009, and International Application No. PCT/US10/28224, filed June 10, 2010, which are hereby incorporated by reference.

ALNY-100 comprising formulations are described, e.g., International patent application number
20 PCT/US09/63933, filed on November 10, 2009, which is hereby incorporated by reference.

C12-200 comprising formulations are described in U.S. Provisional Serial No. 61/175,770, filed May 5, 2009 and International Application No. PCT/US10/33777, filed May 5, 2010, which are hereby incorporated by reference.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitables. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable. In some embodiments, oral formulations are those in which dsRNAs featured in the invention are administered in conjunction with one or more penetration enhancer surfactants and chelators. Suitable surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucolic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprinate, tricaprinate, monoolein, dilaurin, glyceryl 1-monocaprinate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (*e.g.*, sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs featured in the invention can be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pullulans, celluloses and starches. Suitable complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (*e.g.*, p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcyanoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA)), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. Patent 6,887,906, US Publication. No. 20030027780, and U.S. Patent No. 6,747,014, each of which is incorporated herein by reference.

Compositions and formulations for parenteral, intraparenchymal (into the brain), intrathecal, intraventricular or intrahepatic administration can include sterile aqueous solutions which can also contain

buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Particularly preferred are formulations that target the liver when treating hepatic disorders such as chronic inflammation of the liver.

The pharmaceutical formulations of the present invention, which can conveniently be presented in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention can be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention can also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions can further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension can also contain stabilizers.

Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto *et al.* (Bull. Chem. Soc. Jpn., 1980, 53, 2778) described liposomes comprising a nonionic detergent, 2C₁₂15G, that contains a PEG moiety. Illum *et al.* (FEBS Lett., 1984, 167, 79) noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (*e.g.*, PEG) are described by Sears (U.S. Pat. Nos. 4,426,330 and 4,534,899). Klivanov *et al.* (FEBS Lett., 1990, 268, 235) described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume *et al.* (Biochimica et Biophysica Acta, 1990, 1029, 91) extended such observations to other PEG-derivatized phospholipids, *e.g.*, DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle *et al.* (U.S. Pat. Nos. 5,013,556 and 5,356,633) and Martin *et al.* (U.S. Pat. No. 5,213,804 and European Patent No. EP 0 496 813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO

91/05545 and U.S. Pat. No. 5,225,212 (both to Martin *et al.*) and in WO 94/20073 (Zalipsky *et al.*).

Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi *et al.*). U.S. Pat. No. 5,540,935 (Miyazaki *et al.*) and U.S. Pat. No. 5,556,948 (Tagawa *et al.*) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

5 A number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry *et al.* discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Pat. No. 5,264,221 to Tagawa *et al.* discloses protein-bonded liposomes and asserts that the contents of such liposomes may include a dsRNA. U.S. Pat. No. 5,665,710 to Rahman *et al.* describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love *et al.* discloses liposomes
10 comprising dsRNAs targeted to the raf gene.

C. Additional Formulations

i. Emulsions

The compositions of the present invention can be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually
15 exceeding 0.1 μm in diameter (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage
20 Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi *et al.*, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily
25 phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution in either aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants
30 can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise, a system of oil droplets enclosed in globules of
35 water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion can be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants can be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335;

Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that can readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used can be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (see *e.g.*, Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of ease of formulation, as well as efficacy from an absorption and bioavailability standpoint (see *e.g.*, Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

ii. Microemulsions

In one embodiment of the present invention, the compositions of iRNAs and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see *e.g.*, Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in *Pharmaceutical Dosage Forms*,

Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245).

Typically, microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as

5 thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: *Controlled Release of Drugs: Polymers and Aggregate Systems*, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w)
10 type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa., 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (see *e.g.*,
15 *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the
20 advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol
25 pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions can, however, be prepared without
30 the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase can typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase can include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty

alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (see *e.g.*, U.S. Patent Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides *et al.*, Pharmaceutical Research, 1994, 11, 1385-1390; Ritschel, Meth. Find. Exp. Clin. Pharmacol., 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (see *e.g.*, U.S. Patent Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides *et al.*, Pharmaceutical Research, 1994, 11, 1385; Ho *et al.*, J. Pharm. Sci., 1996, 85, 138-143). Often microemulsions can form spontaneously when their components are brought together at ambient temperature. This can be particularly advantageous when formulating thermolabile drugs, peptides or iRNAs. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of iRNAs and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of iRNAs and nucleic acids.

Microemulsions of the present invention can also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the iRNAs and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention can be classified as belonging to one of five broad categories--surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Each of these classes has been discussed above.

iii. Microparticles

An RNAi agent of the invention may be incorporated into a particle, *e.g.*, a microparticle. Microparticles can be produced by spray-drying, but may also be produced by other methods including lyophilization, evaporation, fluid bed drying, vacuum drying, or a combination of these techniques.

iv. Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly iRNAs, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs can cross cell membranes if the membrane

to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers can be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

Surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of iRNAs through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92); and perfluorochemical emulsions, such as FC-43. Takahashi *et al.*, J. Pharm. Pharmacol., 1988, 40, 252).

Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C₁₋₂₀ alkyl esters thereof (*e.g.*, methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (*i.e.*, oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, *etc.*) (see *e.g.*, Toutitou, E., *et al.* Enhancement in Drug Delivery, CRC Press, Danvers, MA, 2006; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri *et al.*, J. Pharm. Pharmacol., 1992, 44, 651-654).

The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman *et al.* Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucolic acid (sodium glucolate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate

(STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783;

5 Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto *et al.*, J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita *et al.*, J. Pharm. Sci., 1990, 79, 579-583).

Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of iRNAs through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention,
10 chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315-339). Suitable chelating agents include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (*e.g.*, sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones
15 (enamines)(see *e.g.*, Katdare, A. *et al.*, Excipient development for pharmaceutical, biotechnology, and drug delivery, CRC Press, Danvers, MA, 2006; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur *et al.*, J. Control Rel., 1990, 14, 43-51).

As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as
20 compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of iRNAs through the alimentary mucosa (see *e.g.*, Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers includes, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as
25 diclofenac sodium, indomethacin and phenylbutazone (Yamashita *et al.*, J. Pharm. Pharmacol., 1987, 39, 621-626).

Agents that enhance uptake of iRNAs at the cellular level can also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi *et al.*, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo
30 *et al.*, PCT Application WO 97/30731), are also known to enhance the cellular uptake of dsRNAs. Examples of commercially available transfection reagents include, for example Lipofectamine™ (Invitrogen; Carlsbad, CA), Lipofectamine 2000™ (Invitrogen; Carlsbad, CA), 293fectin™ (Invitrogen; Carlsbad, CA), Cellfectin™ (Invitrogen; Carlsbad, CA), DMRIE-C™ (Invitrogen; Carlsbad, CA), FreeStyle™ MAX (Invitrogen; Carlsbad, CA), Lipofectamine™ 2000 CD (Invitrogen; Carlsbad, CA), Lipofectamine™ (Invitrogen;
35 Carlsbad, CA), RNAiMAX (Invitrogen; Carlsbad, CA), Oligofectamine™ (Invitrogen; Carlsbad, CA),

Optifect™ (Invitrogen; Carlsbad, CA), X-tremeGENE Q2 Transfection Reagent (Roche; Grenzacherstrasse, Switzerland), DOTAP Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), DOSPER Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), or Fugene (Grenzacherstrasse, Switzerland), Transfectam® Reagent (Promega; Madison, WI), TransFast™ Transfection Reagent (Promega; Madison, WI), Tfx™-20 Reagent (Promega; Madison, WI), Tfx™-50 Reagent (Promega; Madison, WI), DreamFect™ (OZ Biosciences; Marseille, France), EcoTransfect (OZ Biosciences; Marseille, France), TransPass^a D1 Transfection Reagent (New England Biolabs; Ipswich, MA, USA), LyoVec™/LipoGen™ (Invitrogen; San Diego, CA, USA), PerFectin Transfection Reagent (Genlantis; San Diego, CA, USA), NeuroPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), GenePORTER Transfection reagent (Genlantis; San Diego, CA, USA), GenePORTER 2 Transfection reagent (Genlantis; San Diego, CA, USA), Cytofectin Transfection Reagent (Genlantis; San Diego, CA, USA), BaculoPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), TroganPORTER™ transfection Reagent (Genlantis; San Diego, CA, USA), RiboFect (Bioline; Taunton, MA, USA), PlasFect (Bioline; Taunton, MA, USA), UniFECTOR (B-Bridge International; Mountain View, CA, USA), SureFECTOR (B-Bridge International; Mountain View, CA, USA), or HiFect™ (B-Bridge International, Mountain View, CA, USA), among others.

Other agents can be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

v. Carriers

Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, “carrier compound” or “carrier” can refer to a nucleic acid, or analog thereof, which is inert (*i.e.*, does not possess biological activity *per se*) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate dsRNA in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (Miyao *et al.*, DsRNA Res. Dev., 1995, 5, 115-121; Takakura *et al.*, DsRNA & Nucl. Acid Drug Dev., 1996, 6, 177-183).

vi. Excipients

In contrast to a carrier compound, a “pharmaceutical carrier” or “excipient” is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient can be liquid or solid and is selected, with the planned manner of

administration in mind, so as to provide for the desired bulk, consistency, *etc.*, when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (*e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, *etc.*); fillers (*e.g.*, lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, *etc.*); lubricants (*e.g.*, magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, *etc.*); disintegrants (*e.g.*, starch, sodium starch glycolate, *etc.*); and wetting agents (*e.g.*, sodium lauryl sulphate, *etc.*).

Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Formulations for topical administration of nucleic acids can include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions can also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can be used.

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

vii. Other Components

The compositions of the present invention can additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions can contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions can contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension can also contain stabilizers.

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more
5 iRNA compounds and (b) one or more agents which function by a non-RNAi mechanism and which are useful in treating a CIDEB-associated disease, disorder, or condition. Examples of such agents include, but are not limited to pyridoxine, an ACE inhibitor (angiotensin converting enzyme inhibitors), *e.g.*, benazepril (Lotensin); an angiotensin II receptor antagonist (ARB) (*e.g.*, losartan potassium, such as Merck & Co. 's Cozaar®), *e.g.*, Candesartan (Atacand); an HMG-CoA reductase inhibitor (*e.g.*, a statin); calcium binding
10 agents, *e.g.*, Sodium cellulose phosphate (Calcibind); diuretics, *e.g.*, thiazide diuretics, such as hydrochlorothiazide (Microzide); an insulin sensitizer, such as the PPAR γ agonist pioglitazone, a glp-1r agonist, such as liraglutide, vitamin E, an SGLT2 inhibitor, a DPPIV inhibitor, and kidney/liver transplant; or a combination of any of the foregoing.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical
15 procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulating a range of
20 dosage for use in humans. The dosage of compositions featured herein in the invention lies generally within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma
25 concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC₅₀ (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the iRNAs featured in the invention can be
30 administered in combination with other known agents effective in treatment of pathological processes mediated by CIDEB expression. In any event, the administering physician can adjust the amount and timing of iRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

Synthesis of cationic lipids:

Any of the compounds, *e.g.*, cationic lipids and the like, used in the nucleic acid-lipid particles featured in the invention may be prepared by known organic synthesis techniques. All substituents are as defined below unless indicated otherwise.

“Alkyl” means a straight chain or branched, noncyclic or cyclic, saturated aliphatic hydrocarbon containing from 1 to 24 carbon atoms. Representative saturated straight chain alkyls include methyl, ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, *n*-hexyl, and the like; while saturated branched alkyls include isopropyl, *sec*-butyl, isobutyl, *tert*-butyl, isopentyl, and the like. Representative saturated cyclic alkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like; while unsaturated cyclic alkyls include cyclopentenyl and cyclohexenyl, and the like.

“Alkenyl” means an alkyl, as defined above, containing at least one double bond between adjacent carbon atoms. Alkenyls include both *cis* and *trans* isomers. Representative straight chain and branched alkenyls include ethylenyl, propylenyl, 1-butenyl, 2-butenyl, isobutylenyl, 1-pentenyl, 2-pentenyl, 3-methyl-1-butenyl, 2-methyl-2-butenyl, 2,3-dimethyl-2-butenyl, and the like.

“Alkynyl” means any alkyl or alkenyl, as defined above, which additionally contains at least one triple bond between adjacent carbons. Representative straight chain and branched alkynyls include acetylenyl, propynyl, 1-butyne, 2-butyne, 1-pentyne, 2-pentyne, 3-methyl-1-butyne, and the like.

“Acyl” means any alkyl, alkenyl, or alkynyl wherein the carbon at the point of attachment is substituted with an oxo group, as defined below. For example, -C(=O)alkyl , -C(=O)alkenyl , and -C(=O)alkynyl are acyl groups.

“Heterocycle” means a 5- to 7-membered monocyclic, or 7- to 10-membered bicyclic, heterocyclic ring which is either saturated, unsaturated, or aromatic, and which contains from 1 or 2 heteroatoms independently selected from nitrogen, oxygen and sulfur, and wherein the nitrogen and sulfur heteroatoms may be optionally oxidized, and the nitrogen heteroatom may be optionally quaternized, including bicyclic rings in which any of the above heterocycles are fused to a benzene ring. The heterocycle may be attached via any heteroatom or carbon atom. Heterocycles include heteroaryls as defined below. Heterocycles include morpholinyl, pyrrolidinonyl, pyrrolidinyl, piperidinyl, piperizynyl, hydantoinyl, valerolactamyl, oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranlyl, tetrahydropyridinyl, tetrahydroprimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranlyl, tetrahydropyrimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranlyl, and the like.

The terms “optionally substituted alkyl”, “optionally substituted alkenyl”, “optionally substituted alkynyl”, “optionally substituted acyl”, and “optionally substituted heterocycle” means that, when substituted, at least one hydrogen atom is replaced with a substituent. In the case of an oxo substituent (=O) two hydrogen atoms are replaced. In this regard, substituents include oxo, halogen, heterocycle, -CN , -OR^x , $\text{-NR}^x\text{R}^y$, $\text{-NR}^x\text{C(=O)R}^y$, $\text{-NR}^x\text{SO}_2\text{R}^y$, -C(=O)R^x , -C(=O)OR^x , $\text{-C(=O)NR}^x\text{R}^y$, $\text{-SO}_n\text{R}^x$

and $-\text{SO}_n\text{NR}^x\text{R}^y$, wherein n is 0, 1 or 2, R^x and R^y are the same or different and independently hydrogen, alkyl or heterocycle, and each of said alkyl and heterocycle substituents may be further substituted with one or more of oxo, halogen, $-\text{OH}$, $-\text{CN}$, alkyl, $-\text{OR}^x$,

heterocycle, $-\text{NR}^x\text{R}^y$, $-\text{NR}^x\text{C}(=\text{O})\text{R}^y$, $-\text{NR}^x\text{SO}_2\text{R}^y$, $-\text{C}(=\text{O})\text{R}^x$, $-\text{C}(=\text{O})\text{OR}^x$, $-\text{C}(=\text{O})\text{NR}^x\text{R}^y$, $-\text{SO}_n\text{R}^x$

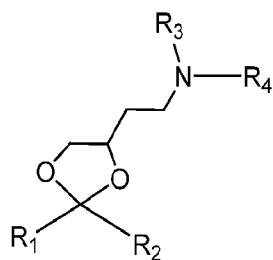
5 and $-\text{SO}_n\text{NR}^x\text{R}^y$.

“Halogen” means fluoro, chloro, bromo and iodo.

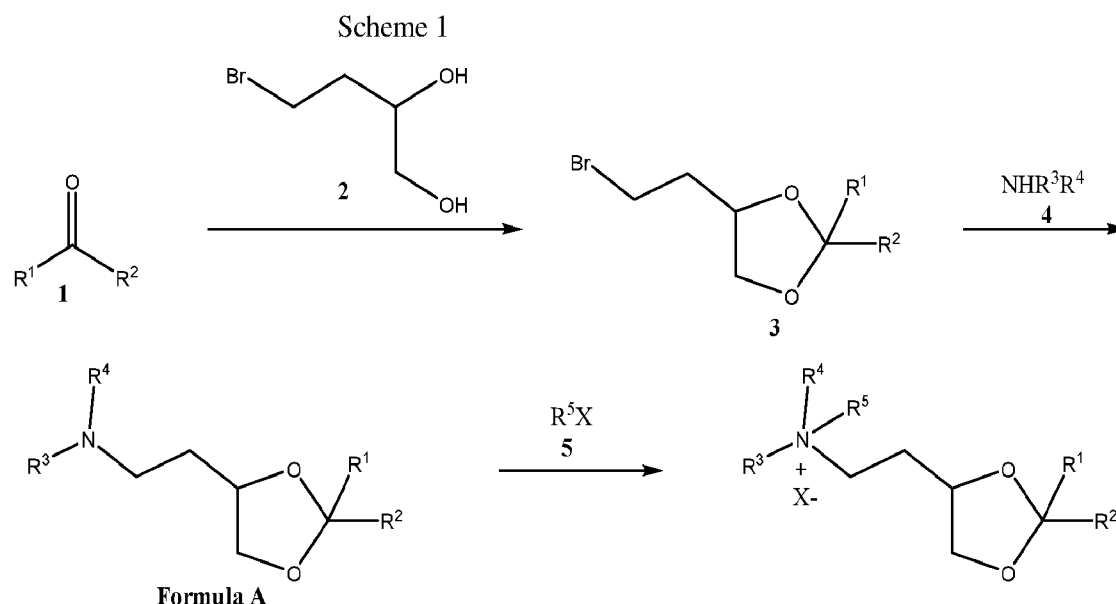
In some embodiments, the methods featured in the invention may require the use of protecting groups. Protecting group methodology is well known to those skilled in the art (*see, for example*, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, Green, T.W. *et al.*, Wiley-Interscience, New York City, 10 1999). Briefly, protecting groups within the context of this invention are any group that reduces or eliminates unwanted reactivity of a functional group. A protecting group can be added to a functional group to mask its reactivity during certain reactions and then removed to reveal the original functional group. In some embodiments an “alcohol protecting group” is used. An “alcohol protecting group” is any group which decreases or eliminates unwanted reactivity of an alcohol functional group. Protecting 15 groups can be added and removed using techniques well known in the art.

Synthesis of Formula A:

In certain embodiments, nucleic acid-lipid particles featured in the invention are formulated using a cationic lipid of formula A:

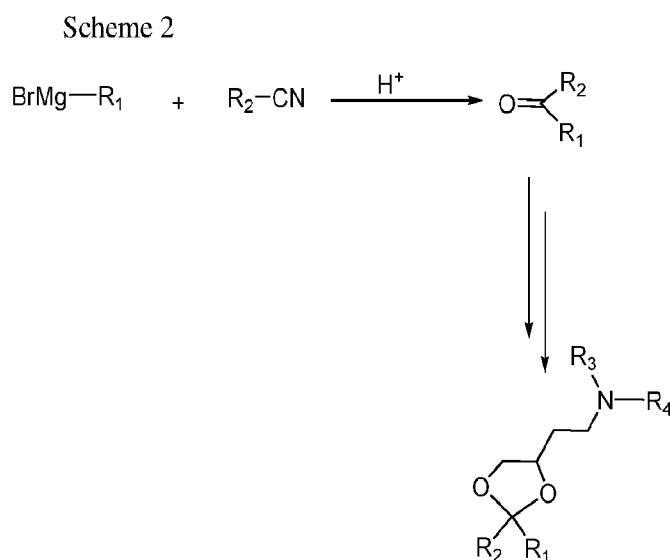


20 where R_1 and R_2 are independently alkyl, alkenyl or alkynyl, each can be optionally substituted, and R_3 and R_4 are independently lower alkyl or R_3 and R_4 can be taken together to form an optionally substituted heterocyclic ring. In some embodiments, the cationic lipid is XTC (2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane). In general, the lipid of formula A above may be made by the following Reaction Schemes 1 or 2, wherein all substituents are as defined above unless indicated 25 otherwise.



Lipid A, where R_1 and R_2 are independently alkyl, alkenyl or alkynyl, each can be optionally substituted, and R_3 and R_4 are independently lower alkyl or R_3 and R_4 can be taken together to form an optionally substituted heterocyclic ring, can be prepared according to Scheme 1. Ketone 1 and bromide 2 can be purchased or prepared according to methods known to those of ordinary skill in the art. Reaction of 1 and 2 yields ketal 3. Treatment of ketal 3 with amine 4 yields lipids of formula A. The lipids of formula A can be converted to the corresponding ammonium salt with an organic salt of formula 5, where

10 X is anion counter ion selected from halogen, hydroxide, phosphate, sulfate, or the like.



Alternatively, the ketone 1 starting material can be prepared according to Scheme 2. Grignard reagent 6 and cyanide 7 can be purchased or prepared according to methods known to those of ordinary skill in the art. Reaction of 6 and 7 yields ketone 1. Conversion of ketone 1 to the corresponding lipids of formula A is as described in Scheme 1.

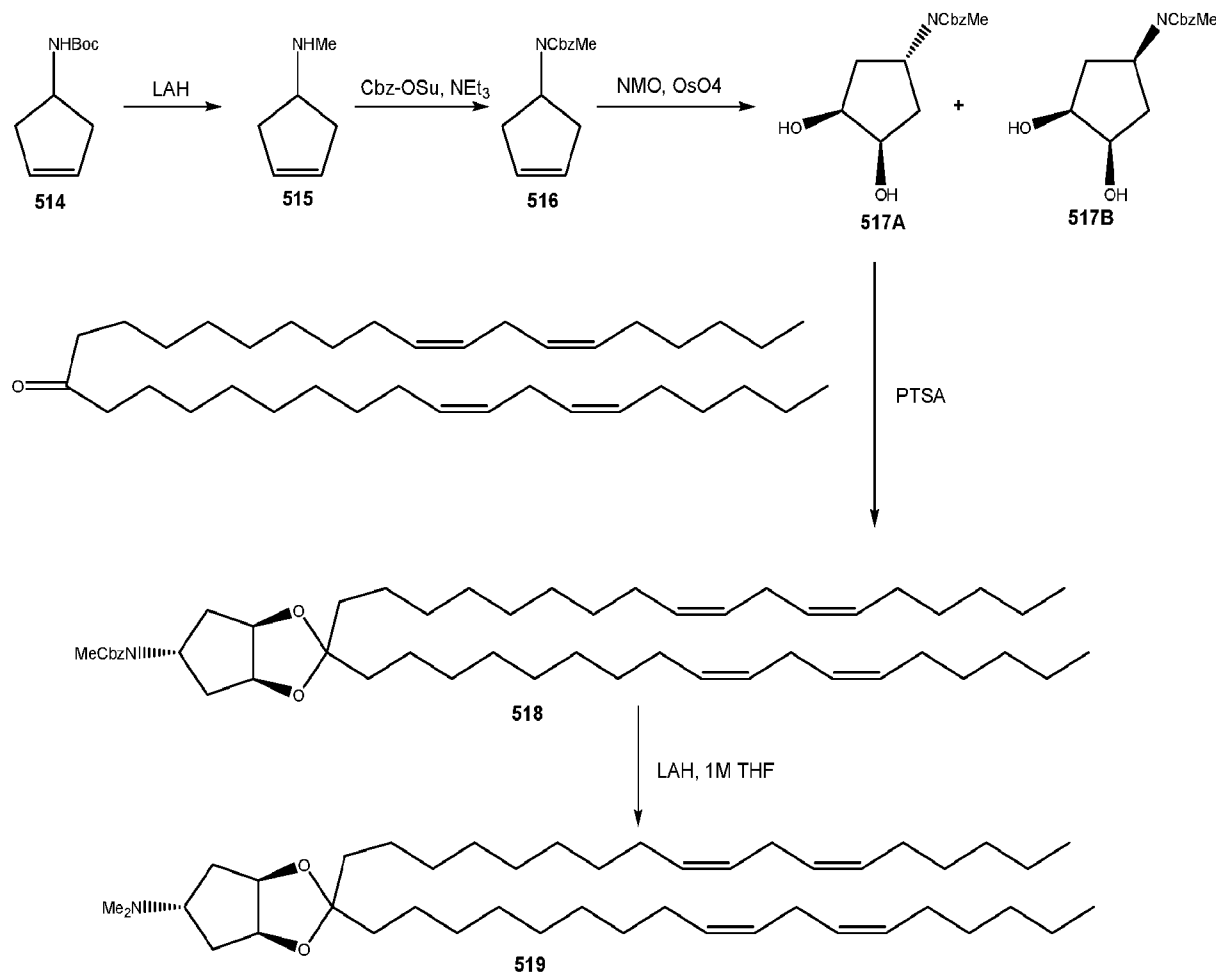
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Synthesis of MC3:

Preparation of DLin-M-C3-DMA (*i.e.*, (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate) was as follows. A solution of (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-ol (0.53 g), 4-N,N-dimethylaminobutyric acid hydrochloride (0.51 g), 4-N,N-dimethylaminopyridine (0.61g) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.53 g) in dichloromethane (5 mL) was stirred at room temperature overnight. The solution was washed with dilute hydrochloric acid followed by dilute aqueous sodium bicarbonate. The organic fractions were dried over anhydrous magnesium sulphate, filtered and the solvent removed on a rotovap. The residue was passed down a silica gel column (20 g) using a 1-5% methanol/dichloromethane elution gradient. Fractions containing the purified product were combined and the solvent removed, yielding a colorless oil (0.54 g).

Synthesis of ALNY-100:

Synthesis of ketal 519 [ALNY-100] was performed using the following scheme 3:



Synthesis of 515:

To a stirred suspension of LiAlH_4 (3.74 g, 0.09852 mol) in 200 ml anhydrous THF in a two neck RBF (1L), was added a solution of 514 (10g, 0.04926mol) in 70 mL of THF slowly at 0 °C under nitrogen atmosphere. After complete addition, reaction mixture was warmed to room temperature and then heated to reflux for 4 h. Progress of the reaction was monitored by TLC. After completion of reaction (by TLC) the mixture was cooled to 0 °C and quenched with careful addition of saturated Na_2SO_4 solution. Reaction mixture was stirred for 4 h at room temperature and filtered off. Residue was washed well with THF. The filtrate and washings were mixed and diluted with 400 mL dioxane and 26 mL conc. HCl and stirred for 20 minutes at room temperature. The volatilities were stripped off under vacuum to furnish the hydrochloride salt of 515 as a white solid. Yield: 7.12 g $^1\text{H-NMR}$ (DMSO , 400MHz): δ = 9.34 (broad, 2H), 5.68 (s, 2H), 3.74 (m, 1H), 2.66-2.60 (m, 2H), 2.50-2.45 (m, 5H).

Synthesis of 516:

To a stirred solution of compound 515 in 100 mL dry DCM in a 250 mL two neck RBF, was added NEt_3 (37.2 mL, 0.2669 mol) and cooled to 0 °C under nitrogen atmosphere. After a slow addition of N-(benzyloxy-carbonyloxy)-succinimide (20 g, 0.08007 mol) in 50 mL dry DCM, reaction mixture was allowed to warm to room temperature. After completion of the reaction (2-3 h by TLC) mixture was washed successively with 1N HCl solution (1 x 100 mL) and saturated NaHCO_3 solution (1 x 50 mL). The organic layer was then dried over anhyd. Na_2SO_4 and the solvent was evaporated to give crude material which was purified by silica gel column chromatography to get 516 as sticky mass. Yield: 11g (89%). $^1\text{H-NMR}$ (CDCl_3 , 400MHz): δ = 7.36-7.27(m, 5H), 5.69 (s, 2H), 5.12 (s, 2H), 4.96 (br., 1H) 2.74 (s, 3H), 2.60(m, 2H), 2.30-2.25(m, 2H). LC-MS $[\text{M}+\text{H}]^-$ -232.3 (96.94%).

Synthesis of 517A and 517B:

The cyclopentene 516 (5 g, 0.02164 mol) was dissolved in a solution of 220 mL acetone and water (10:1) in a single neck 500 mL RBF and to it was added N-methyl morpholine-N-oxide (7.6 g, 0.06492 mol) followed by 4.2 mL of 7.6% solution of OsO_4 (0.275 g, 0.00108 mol) in tert-butanol at room temperature. After completion of the reaction (~ 3 h), the mixture was quenched with addition of solid Na_2SO_3 and resulting mixture was stirred for 1.5 h at room temperature. Reaction mixture was diluted with DCM (300 mL) and washed with water (2 x 100 mL) followed by saturated NaHCO_3 (1 x 50 mL) solution, water (1 x 30 mL) and finally with brine (1x 50 mL). Organic phase was dried over Na_2SO_4 and solvent was removed in vacuum. Silica gel column chromatographic purification of the crude material was afforded a mixture of diastereomers, which were separated by prep HPLC. Yield: ~ 6 g crude 517A - Peak-1 (white solid), 5.13 g (96%). $^1\text{H-NMR}$ (DMSO , 400MHz): δ = 7.39-7.31(m, 5H),

5.04(s, 2H), 4.78-4.73 (m, 1H), 4.48-4.47(d, 2H), 3.94-3.93(m, 2H), 2.71(s, 3H), 1.72- 1.67(m, 4H). LC-MS - [M+H]⁺-266.3, [M+NH₄]⁺-283.5 present, HPLC-97.86%. Stereochemistry confirmed by X-ray.

Synthesis of 518:

5 Using a procedure analogous to that described for the synthesis of compound 505, compound 518 (1.2 g, 41%) was obtained as a colorless oil. ¹H-NMR (CDCl₃, 400MHz): δ= 7.35-7.33(m, 4H), 7.30-7.27(m, 1H), 5.37-5.27(m, 8H), 5.12(s, 2H), 4.75(m, 1H), 4.58-4.57(m, 2H), 2.78-2.74(m, 7H), 2.06-2.00(m, 8H), 1.96-1.91(m, 2H), 1.62(m, 4H), 1.48(m, 2H), 1.37-1.25(br m, 36H), 0.87(m, 6H). HPLC-98.65%.

General Procedure for the Synthesis of Compound 519:

10 A solution of compound 518 (1 eq) in hexane (15 mL) was added in a drop-wise fashion to an ice-cold solution of LAH in THF (1 M, 2 eq). After complete addition, the mixture was heated at 40°C over 0.5 h then cooled again on an ice bath. The mixture was carefully hydrolyzed with saturated aqueous Na₂SO₄ then filtered through Celite® and reduced to an oil. Column chromatography provided the pure 519 (1.3 g, 68%) which was obtained as a colorless oil. ¹³C NMR = 130.2, 130.1 (x2), 127.9 (x3), 112.3, 79.3, 64.4, 44.7, 38.3, 35.4, 31.5, 29.9 (x2), 29.7, 29.6 (x2), 29.5 (x3), 29.3 (x2), 27.2 (x3), 25.6, 24.5, 23.3, 22.6, 14.1; Electrospray MS (+ve): Molecular weight for C₄₄H₈₀NO₂ (M + H)⁺ Calc. 654.6, Found 654.6.

20 Formulations prepared by either the standard or extrusion-free method can be characterized in similar manners. For example, formulations are typically characterized by visual inspection. They should be whitish translucent solutions free from aggregates or sediment. Particle size and particle size distribution of lipid-nanoparticles can be measured by light scattering using, for example, a Malvern Zetasizer Nano ZS (Malvern, USA). Particles should be about 20-300 nm, such as 40-100 nm in size.

25 The particle size distribution should be unimodal. The total dsRNA concentration in the formulation, as well as the entrapped fraction, is estimated using a dye exclusion assay. A sample of the formulated dsRNA can be incubated with an RNA-binding dye, such as RiboGreen® (Molecular Probes) in the presence or absence of a formulation disrupting surfactant, *e.g.*, 0.5% Triton-X100. The total dsRNA in the formulation can be determined by the signal from the sample containing the surfactant, relative to a standard curve. The entrapped fraction is determined by subtracting the “free” dsRNA content (as measured by the signal in the absence of surfactant) from the total dsRNA content. Percent entrapped dsRNA is typically >85%. For SNALP formulation, the particle size is at least 30 nm, at least 40 nm, at least 50 nm, at least 60 nm, at least 70 nm, at least 80 nm, at least 90 nm, at least 100 nm, at least 110 nm, and at least 120 nm. The suitable range is typically about at least 50 nm to about at least 110 nm, about at least 60 nm to about at least 100 nm, or about at least 80 nm to about at least 90 nm.

VII. Methods of the Invention

The present invention also provides methods of using an iRNA of the invention and/or a composition of the invention to reduce and/or inhibit CIDEB expression in a cell, such as a cell in a subject, *e.g.*, a hepatocyte. The methods include contacting the cell with an RNAi agent or pharmaceutical composition comprising an iRNA agent of the invention. In some embodiments, the cell is maintained for a time sufficient to obtain degradation of the mRNA transcript of a CIDEB gene.

The present invention also provides methods of using an iRNA of the invention and/or a composition of the invention and an iRNA agent targeting a Patatin-like Phospholipase Domain Containing 3 (PNPLA3) gene and/or pharmaceutical composition comprising an iRNA agent targeting PNPLA3 to reduce and/or inhibit PNPLA3 expression in a cell, such as a cell in a subject, *e.g.*, a hepatocyte.

In addition, the present invention provides methods of inhibiting the accumulation and/or expansion of lipid droplets in a cell, such as a cell in a subject, *e.g.*, a hepatocyte. The methods include contacting the cell with an RNAi agent or pharmaceutical composition comprising an iRNA agent of the invention and an iRNA agent targeting a PNPLA3 gene and/or pharmaceutical composition comprising an iRNA agent targeting PNPLA3. In some embodiments, the cell is maintained for a time sufficient to obtain degradation of the mRNA transcript of a CIDEB gene and a PNPLA3 gene.

Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of CIDEB may be determined by determining the mRNA expression level of CIDEB using methods routine to one of ordinary skill in the art, *e.g.*, Northern blotting, qRT-PCR; by determining the protein level of CIDEB using methods routine to one of ordinary skill in the art, such as Western blotting, immunological techniques. A reduction in the expression of CIDEB may also be assessed indirectly by measuring a decrease in biological activity of CIDEB, *e.g.*, a decrease in the interaction of CIDEB with ApoB and/or a decrease in lipid maturation in the liver.

Suitable agents targeting a PNPLA3 gene are described in, for example, U.S. Patent Publication No.: 2017/0340661, the entire contents of which are incorporated herein by reference.

In the methods of the invention the cell may be contacted *in vitro* or *in vivo*, *i.e.*, the cell may be within a subject.

A cell suitable for treatment using the methods of the invention may be any cell that expresses a CIDEB gene (and, in some embodiments, a PNPLA3 gene). A cell suitable for use in the methods of the invention may be a mammalian cell, *e.g.*, a primate cell (such as a human cell or a non-human primate cell, *e.g.*, a monkey cell or a chimpanzee cell), a non-primate cell (such as a cow cell, a pig cell, a camel cell, a llama cell, a horse cell, a goat cell, a rabbit cell, a sheep cell, a hamster, a guinea pig cell, a cat cell, a dog cell, a rat cell, a mouse cell, a lion cell, a tiger cell, a bear cell, or a buffalo cell), a bird cell (*e.g.*, a duck cell or a goose cell), or a whale cell. In one embodiment, the cell is a human cell, *e.g.*, a human liver cell.

CIDEB expression is inhibited in the cell by at least about 5, 6, 7, 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, 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, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or about 100%. In

5 preferred embodiments, CIDEB expression is inhibited by at least 20%.

In some embodiment, PNPLA3 expression is also inhibited in the cell by at least about 5, 6, 7, 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, 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, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or about 100%. In preferred embodiments, PNPLA3 expression is inhibited by at least 20%.

10 In one embodiment, the *in vivo* methods of the invention may include administering to a subject a composition containing an iRNA, where the iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the CIDEB gene of the mammal to be treated.

In another embodiment, the *in vivo* methods of the invention may include administering to a subject a composition containing a first iRNA agent and a second iRNA agent, where the first iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the CIDEB gene of the mammal to be treated and the second iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the PNPLA3 gene of the mammal to be treated.

15 When the organism to be treated is a mammal such as a human, the composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (*e.g.*, intraventricular, intraparenchymal and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection.

20 In some embodiments, the administration is *via* a depot injection. A depot injection may release the iRNA in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, *e.g.*, a desired inhibition of CIDEB, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

25 In some embodiments, the administration is *via* a pump. The pump may be an external pump or a surgically implanted pump. In certain embodiments, the pump is a subcutaneously implanted osmotic pump. In other embodiments, the pump is an infusion pump. An infusion pump may be used for intravenous, subcutaneous, arterial, or epidural infusions. In preferred embodiments, the infusion pump is a subcutaneous

infusion pump. In other embodiments, the pump is a surgically implanted pump that delivers the iRNA to the liver.

An iRNA of the invention may be present in a pharmaceutical composition, such as in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution containing the iRNA can be adjusted such that it is suitable for administering to a subject.

Alternatively, an iRNA of the invention may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.

In one aspect, the present invention also provides methods for inhibiting the expression of a CIDEB gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets a CIDEB gene in a cell of the mammal, thereby inhibiting expression of the CIDEB gene in the cell.

In some embodiments, the methods include administering to the mammal a composition comprising a dsRNA that targets a CIDEB gene in a cell of the mammal, thereby inhibiting expression of the CIDEB gene in the cell. In another embodiment, the methods include administering to the mammal a pharmaceutical composition comprising a dsRNA agent that targets a CIDEB gene in a cell of the mammal.

In another aspect, the present invention provides use of an iRNA agent or a pharmaceutical composition of the invention for inhibiting the expression of a CIDEB gene in a mammal.

In yet another aspect, the present invention provides use of an iRNA agent of the invention targeting a CIDEB gene or a pharmaceutical composition comprising such an agent in the manufacture of a medicament for inhibiting expression of a CIDEB gene in a mammal.

In another aspect, the present invention also provides methods for inhibiting the expression of a CIDEB gene and a PNPLA3 gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets a CIDEB gene in a cell of the mammal and a composition comprising a dsRNA that targets a PNPLA3 gene in a cell of the mammal, thereby inhibiting expression of the CIDEB gene and the PNPLA3 gene in the cell. In one embodiment, the methods include administering to the mammal a pharmaceutical composition comprising a dsRNA agent that targets a CIDEB gene and a PNPLA3 gene in a cell of the mammal.

In one aspect, the present invention provides use of an iRNA agent or a pharmaceutical composition of the invention, and a dsRNA that targets a PNPLA3 gene or a pharmaceutical composition comprising such an agent for inhibiting the expression of a CIDEB gene and a PNPLA3 gene in a mammal.

In yet another aspect, the present invention provides use of an iRNA agent of the invention targeting a CIDEB gene or a pharmaceutical composition comprising such an agent, and a dsRNA that targets an PNPLA3 gene or a pharmaceutical composition comprising such an agent in the manufacture of a medicament for inhibiting expression of a CIDEB gene and a PNPLA3 gene in a mammal.

5 Reduction in gene expression can be assessed by any methods known in the art and by methods, *e.g.* qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known in the art and by methods, *e.g.* ELISA, enzymatic activity, described herein.

The present invention also provides therapeutic and prophylactic methods which include administering to a subject having, or prone to developing a fatty liver-associated disease, disorder, or
10 condition, the iRNA agents, pharmaceutical compositions comprising an iRNA agent, or vectors comprising an iRNA of the invention.

In one aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in CIDEB expression, *e.g.*, a CIDEB-associated disease.

The treatment methods (and uses) of the invention include administering to the subject, *e.g.*, a human,
15 a therapeutically effective amount of a dsRNA agent that inhibits expression of CIDEB or a pharmaceutical composition comprising a dsRNA that inhibits expression of CIDEB, thereby treating the subject.

In one aspect, the invention provides methods of preventing at least one symptom in a subject having a disorder that would benefit from reduction in CIDEB expression, *e.g.*, a chronic inflammatory disease. The methods include administering to the subject a prophylactically effective amount of dsRNA agent or a
20 pharmaceutical composition comprising a dsRNA, thereby preventing at least one symptom in the subject.

In one embodiment, a CIDEB-associated disease, disorder, or condition is a chronic inflammatory disease. Non-limiting examples of chronic inflammatory diseases include inflammation of the liver and other tissues. Non-limiting examples of chronic inflammatory liver diseases include liver fibrosis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), cirrhosis of the liver, alcoholic
25 steatohepatitis (ASH), alcoholic liver diseases (ALD), HCV-associated cirrhosis, drug induced liver injury, hepatocellular necrosis, hepatocellular carcinoma, insulin insensitivity and/or diabetes.

The present invention also provides therapeutic and prophylactic methods which include administering to a subject having, or prone to developing a fatty liver-associated disease, disorder, or condition, the iRNA agents, pharmaceutical compositions comprising an iRNA agent, or vectors comprising
30 an iRNA of the invention and iRNA agent targeting PNPLA3, pharmaceutical compositions comprising such an iRNA agent, or vectors comprising such an iRNA.

The present invention also provides use of a therapeutically effective amount of an iRNA agent of the invention or a pharmaceutical composition comprising a dsRNA that inhibits expression of CIDEB for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of CIDEB expression,
35 *e.g.*, a CIDEB-associated disease, *e.g.*, a chronic inflammatory disease. Non-limiting examples of chronic

inflammatory liver diseases include liver fibrosis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), cirrhosis of the liver, alcoholic steatohepatitis (ASH), alcoholic liver diseases (ALD), HCV-associated cirrhosis, drug induced liver injury, hepatocellular necrosis, hepatocellular carcinoma, insulin insensitivity and/or diabetes.

5 In another aspect, the present invention provides use of an iRNA agent, *e.g.*, a dsRNA, of the invention targeting a CIDEB for gene or a pharmaceutical composition comprising an iRNA agent targeting a CIDEB for gene in the manufacture of a medicament for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of CIDEB for expression, *e.g.*, a CIDEB-associated disease.

10 The present invention also provides use of a prophylactically effective amount of an iRNA agent of the invention or a pharmaceutical composition comprising a dsRNA that inhibits expression of CIDEB for preventing at least one symptom in a subject having a disorder that would benefit from reduction in CIDEB expression, *e.g.*, a chronic inflammatory disease. Non-limiting examples of chronic inflammatory liver diseases include liver fibrosis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), cirrhosis of the liver, alcoholic steatohepatitis (ASH), alcoholic liver diseases (ALD), HCV-
15 associated cirrhosis, drug induced liver injury, hepatocellular necrosis, hepatocellular carcinoma, insulin insensitivity and/or diabetes.

In another aspect, the present invention provides use of an iRNA agent, *e.g.*, a dsRNA, of the invention targeting a CIDEB gene or a pharmaceutical composition comprising an iRNA agent targeting a CIDEB gene in the manufacture of a medicament for preventing at least one symptom in a subject having a
20 disorder that would benefit from reduction in CIDEB expression, *e.g.*, a chronic inflammatory disease. Non-limiting examples of chronic inflammatory liver diseases include liver fibrosis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), cirrhosis of the liver, alcoholic steatohepatitis (ASH), alcoholic liver diseases (ALD), HCV-associated cirrhosis, drug induced liver injury, hepatocellular necrosis, hepatocellular carcinoma, insulin insensitivity and/or diabetes.

25 In one aspect, the present invention also provides use of a therapeutically effective amount of an iRNA agent of the invention or a pharmaceutical composition comprising a dsRNA that inhibits expression of CIDEB in combination with a dsRNA that targets a PNPLA3 gene or a pharmaceutical composition comprising such an agent for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of CIDEB expression, *e.g.*, a CIDEB-associated disease, *e.g.*, a chronic inflammatory disease. Non-
30 limiting examples of chronic inflammatory liver diseases include liver fibrosis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), cirrhosis of the liver, alcoholic steatohepatitis (ASH), alcoholic liver diseases (ALD), HCV-associated cirrhosis, drug induced liver injury, hepatocellular necrosis, hepatocellular carcinoma, insulin insensitivity and/or diabetes.

35 In one aspect, the present invention also provides use of an iRNA agent, *e.g.*, a dsRNA, of the invention targeting a CIDEB gene or a pharmaceutical composition comprising an iRNA agent targeting a

CIDEB gene in combination with a dsRNA that targets a PNPLA3 gene or a pharmaceutical composition comprising such an agent for preventing at least one symptom in a subject having a disorder that would benefit from reduction in CIDEB expression, *e.g.*, a chronic inflammatory disease. Non-limiting examples of chronic inflammatory liver diseases include liver fibrosis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), cirrhosis of the liver, alcoholic steatohepatitis (ASH), alcoholic liver diseases (ALD), HCV-associated cirrhosis, drug induced liver injury, hepatocellular necrosis, hepatocellular carcinoma, insulin insensitivity and/or diabetes.

The combination methods of the invention for treating a subject, *e.g.*, a human subject, having a CIDEB-associated disease, disorder, or condition, such as a chronic inflammatory disease, *e.g.*, chronic inflammatory liver disease, *e.g.*, NASH, are useful for treating such subjects as silencing of PNPLA3 decreases steatosis (*i.e.* liver fat).

Accordingly, in one aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in CIDEB expression, *e.g.*, a CIDEB-associated disease, such as a chronic inflammatory disease (*e.g.*, inflammation of the liver and other tissues). In one embodiment, the chronic inflammatory disease is chronic inflammatory liver disease (*e.g.*, liver fibrosis, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), cirrhosis of the liver, alcoholic steatohepatitis (ASH), alcoholic liver diseases (ALD), HCV-associated cirrhosis, drug induced liver injury, hepatocellular necrosis, and/or hepatocellular carcinoma). In one embodiment, the chronic inflammatory liver disease is NASH.

The combination treatment methods (and uses) of the invention include administering to the subject, *e.g.*, a human subject, a therapeutically effective amount of a dsRNA agent that inhibits expression of CIDEB or a pharmaceutical composition comprising a dsRNA that inhibits expression of CIDEB, and a dsRNA agent that inhibits expression of PNPLA3 or a pharmaceutical composition comprising a dsRNA that inhibits expression of PNPLA3, thereby treating the subject.

In one aspect, the invention provides methods of preventing at least one symptom in a subject having a disorder that would benefit from reduction in CIDEB expression, *e.g.*, a chronic inflammatory disease. The methods include administering to the subject a prophylactically effective amount of dsRNA agent or a pharmaceutical composition comprising a dsRNA that inhibits expression of CIDEB, and a dsRNA agent that inhibits expression of PNPLA3 or a pharmaceutical composition comprising a dsRNA that inhibits expression of PNPLA3, thereby preventing at least one symptom in the subject.

In one embodiment, the subject is heterozygous for the gene encoding the patatin like phospholipase domain containing 3 (PNPLA3) I148M variation, that is, the subject may have an allele of the gene encoding a PNPLA3 I148M variant and the other allele encoding a different variant. In another embodiment, the subject is homozygous for the gene encoding the PNPLA3 I148M variation, that is, both alleles of the gene encode PNPLA3 I148M variant. In one embodiment, the subject is heterozygous for the gene encoding the patatin

like phospholipase domain containing 3 (PNPLA3) I144M variation, that is, the subject may have an allele of the gene encoding a PNPLA3 I144M variant and the other allele encoding a different variant. In another embodiment, the subject is homozygous for the gene encoding the PNPLA3 I144M variation, that is, both alleles of the gene encode PNPLA3 I143M variant.

5 In certain embodiments of the invention the methods may include identifying a subject that would benefit from reduction in CIDEA expression. The methods generally include determining whether or not a sample from the subject comprises a nucleic acid encoding a PNPLA3Ile148Met variant or a PNPLA3Ile144Met variant. The methods may also include classifying a subject as a candidate for treating or inhibiting a liver disease by inhibiting the expression of CIDEA, by determining whether or not a sample from
10 the subject comprises a first nucleic acid encoding a PNPLA3 protein comprising an I148M variation and a second nucleic acid encoding a functional CIDEA protein, and/or a PNPLA3 protein comprising an I144M variation and a functional CIDEA protein, and classifying the subject as a candidate for treating or inhibiting a liver disease by inhibiting CIDEA when both the first and second nucleic acids are detected and/or when both proteins are detected.

15 The variant PNPLA3 Ile148Met variant or PNPLA3 Ile144Met variant can be any of the PNPLA3 Ile148Met variants and PNPLA3 Ile144Met variants described herein. The PNPLA3 Ile148Met variant or PNPLA3 Ile144Met variant can be detected by any suitable means, such as ELISA assay, RT-PCR, sequencing.

In some embodiments, the methods further comprise determining whether the subject is homozygous
20 or heterozygous for the PNPLA3 Ile148Met variant or the PNPLA3 Ile144Met variant. In some embodiments, the subject is homozygous for the PNPLA3 Ile148Met variant or the PNPLA3 Ile144Met variant. A subject homozygous for the PNPLA3 Ile148Met variant has both alleles of the gene encoding PNPLA3 Ile148Met variant; a subject homozygous for the PNPLA3 Ile144Met variant has both alleles of the gene encoding PNPLA3 Ile144Met variant. In some embodiments, the subject is heterozygous for the PNPLA3 Ile148Met
25 variant or the PNPLA3 Ile144Met variant. A subject heterozygous for the PNPLA3 Ile148Met variant may have an allele for the gene encoding for PNPLA3 Ile148Met variant and the other allele encoding for a different PNPLA3 variant; a subject heterozygous for the PNPLA3 Ile144Met variant may have an allele for the gene encoding for PNPLA3 Ile144Met variant and the other allele encoding for a different PNPLA3 variant. In some embodiments, the subject is homozygous for the PNPLA3 Ile148Met variant. In some
30 embodiments, the subject is heterozygous for the PNPLA3 Ile148Met variant. In some embodiments, the subject is homozygous for the PNPLA3 Ile144Met variant. In some embodiments, the subject is heterozygous for the PNPLA3 Ile144Met variant.

In some embodiments, the methods further comprise determining whether the subject is obese. In some embodiments, a subject is obese if their body mass index (BMI) is over 30 kg/m². Obesity can be a
35 characteristic of a subject having, or at risk of developing, a liver disease. In some embodiments, the methods

further comprise determining whether the subject has a fatty liver. A fatty liver can be a characteristic of a subject having, or at risk of developing, a liver disease. In some embodiments, the methods further comprise determining whether the subject is obese and has a fatty liver.

As used herein, “nonalcoholic fatty liver disease,” used interchangeably with the term “NAFLD,” refers to a disease defined by the presence of macrovascular steatosis in the presence of less than 20 gm of alcohol ingestion per day. NAFLD is the most common liver disease in the United States, and is commonly associated with insulin resistance/type 2 diabetes mellitus and obesity. NAFLD is manifested by steatosis, steatohepatitis, cirrhosis, and sometimes hepatocellular carcinoma. For a review of NAFLD, see Tolman and Dalpiaz (2007) *Ther. Clin. Risk. Manag.*, 3(6):1153-1163 the entire contents of which are incorporated herein by reference.

As used herein, the terms “steatosis,” “hepatic steatosis,” and “fatty liver disease” refer to the accumulation of triglycerides and other fats in the liver cells.

As used herein, the term “NAFLD” refers to non-alcoholic fatty liver disease. NAFLD is the commonest form of liver disease in all regions of the world with modern industrialized economies, including Korea and many other Asian countries. Patients usually present without symptoms or clinical features are non-specific. Instead, liver abnormalities are found incidentally by hepatic imaging, particularly ultrasonography, and/or there are raised liver enzymes (alanine aminotransferase [ALT] and gamma-glutamyltranspeptidase). The diagnosis of NAFLD requires exclusion of other disorders, particularly viral hepatitis, significant alcohol intake, and exposure to potentially hepatotoxic medications. By agreements such as the Asia-Pacific Guidelines on NAFLD, the term NAFLD is now retained for cases of fatty liver associated with metabolic complications of over-nutrition, usually with central obesity and overweight (Farrell *et al.*, *Gut Liver*. 6(2): 149–171, 2012).

As used herein, the term “Nonalcoholic steatohepatitis” or “NASH” refers to liver inflammation and damage caused by a buildup of fat in the liver. NASH is considered the progressive form of nonalcoholic fatty liver disease (NAFLD) and is characterized by liver steatosis, inflammation, hepatocellular injury and different degrees of fibrosis. Adipose tissue dysfunction and the hepatic inflammatory response have a fundamental role during NASH development. Cellular and molecular response mechanisms also promote liver inflammation in the absence of a fatty liver by inducing a chronic inflammatory response that results in hepatocyte damage.

NASH resembles alcoholic liver disease, but occurs in people who drink little or no alcohol. The major feature in NASH is fat in the liver, along with inflammation and damage. Most people with NASH feel well and are not aware that they have a liver problem. Nevertheless, NASH can be severe and can lead to cirrhosis, in which the liver is permanently damaged and scarred and no longer able to work properly. NASH is usually first suspected in a person who is found to have elevations in liver tests that are included in routine blood test panels, such as alanine aminotransferase (ALT) or aspartate aminotransferase (AST). When further

evaluation shows no apparent reason for liver disease (such as medications, viral hepatitis, or excessive use of alcohol) and when x rays or imaging studies of the liver show fat, NASH is suspected. The only means of proving a diagnosis of NASH and separating it from simple fatty liver is a liver biopsy.

As used herein, the term “cirrhosis,” defined histologically, is a diffuse hepatic process characterized by fibrosis and conversion of the normal liver architecture into structurally abnormal nodules.

As used herein, the term “serum lipid” refers to any major lipid present in the blood. Serum lipids may be present in the blood either in free form or as a part of a protein complex, *e.g.*, a lipoprotein complex. Non-limiting examples of serum lipids may include triglycerides (TG), cholesterol, such as total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C) and intermediate-density lipoprotein cholesterol (IDL-C).

In one embodiment, a subject that would benefit from the reduction of the expression of CIDEA (and, in some embodiments, PNPLA3) is, for example, a subject that has type 2 diabetes and prediabetes, or obesity; a subject that has high levels of fats in the blood, such as cholesterol, or has high blood pressure; a subject that has certain metabolic disorders, including metabolic syndrome; a subject that has rapid weight loss; a subject that has certain infections, such as hepatitis C infection, or a subject that has been exposed to some toxins. In one embodiment, a subject that would benefit from the reduction of the expression of CIDEA (and, in some embodiments, PNPLA3) is, for example, a subject that is middle-aged or older; a subject that is Hispanic, non-Hispanic whites, or African Americans; a subject that takes certain drugs, such as corticosteroids and cancer drugs.

In the methods (and uses) of the invention which comprise administering to a subject a first dsRNA agent targeting CIDEA and a second dsRNA agent targeting PNPLA3, the first and second dsRNA agents may be formulated in the same composition or different compositions and may administered to the subject in the same composition or in separate compositions.

In one embodiment, an “iRNA” for use in the methods of the invention is a “dual targeting RNAi agent.” The term “dual targeting RNAi agent” refers to a molecule comprising a first dsRNA agent comprising a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having “sense” and “antisense” orientations with respect to a first target RNA, *i.e.*, a CIDEA gene, covalently attached to a molecule comprising a second dsRNA agent comprising a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having “sense” and “antisense” orientations with respect to a second target RNA, *i.e.*, a PNPLA3 gene. In some embodiments of the invention, a dual targeting RNAi agent triggers the degradation of the first and the second target RNAs, *e.g.*, mRNAs, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

The dsRNA agent may be administered to the subject at a dose of about 0.1 mg/kg to about 50 mg/kg. Typically, a suitable dose will be in the range of about 0.1 mg/kg to about 5.0 mg/kg, preferably about 0.3 mg/kg and about 3.0 mg/kg.

The iRNA can be administered by intravenous infusion over a period of time, on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis.

Administration of the iRNA can reduce CIDEB levels, *e.g.*, in a cell, tissue, blood, urine or other compartment of the patient by at least about 5%, 6, 7, 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, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or at least about 99% or more. In a preferred embodiment, administration of the iRNA can reduce CIDEB levels, *e.g.*, in a cell, tissue, blood, urine or other compartment of the patient by at least 20%.

Administration of the iRNA can reduce PNPLA3 levels, *e.g.*, in a cell, tissue, blood, urine or other compartment of the patient by at least about 5%, 6, 7, 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, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or at least about 99% or more. In a preferred embodiment, administration of the iRNA can reduce PNPLA3 levels, *e.g.*, in a cell, tissue, blood, urine or other compartment of the patient by at least 20%.

Before administration of a full dose of the iRNA, patients can be administered a smaller dose, such as a 5% infusion reaction, and monitored for adverse effects, such as an allergic reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (*e.g.*, TNF-alpha or INF-alpha) levels.

Alternatively, the iRNA can be administered subcutaneously, *i.e.*, by subcutaneous injection. One or more injections may be used to deliver the desired daily dose of iRNA to a subject. The injections may be repeated over a period of time. The administration may be repeated on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as every other day or to once a year. In certain embodiments, the iRNA is administered about once per week, once every 7-10 days, once every 2 weeks, once every 3 weeks, once every 4 weeks, once every 5 weeks, once every 6 weeks, once every 7 weeks, once every 8 weeks, once every 9 weeks, once every 10 weeks, once every 11 weeks, once every 12 weeks, once per month, once every 2 months, once every 3 months, once per quarter), once every 4 months, once every 5 months, or once every 6 months.

In one embodiment, the method includes administering a composition featured herein such that expression of the target CIDEB gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 18, 24 hours, 28, 32, or about 36 hours. In one embodiment, expression of the target CIDEB gene is decreased for an extended duration, *e.g.*, at least about two, three, four days or more, *e.g.*, about one week, two weeks, three weeks, or four weeks or longer.

In another embodiment, the method includes administering a composition featured herein such that expression of the target PNPLA3 gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 18, 24 hours, 28, 32, or about 36 hours. In one embodiment, expression of the target PNPLA3 gene is decreased for an extended duration, *e.g.*, at least about two, three, four days or more, *e.g.*, about one week, two weeks, three weeks, or four weeks or longer.

Preferably, the iRNAs useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target CIDEB gene (and, in some embodiments, a PNPLA3 gene). Compositions and methods for inhibiting the expression of these genes using iRNAs can be prepared and performed as described herein.

Administration of the dsRNA according to the methods of the invention may result in a reduction of the severity, signs, symptoms, and/or markers of such diseases or disorders in a patient with a disorder of lipid metabolism. By "reduction" in this context is meant a statistically significant decrease in such level. The reduction can be, for example, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 100%.

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of a disorder of lipid metabolism may be assessed, for example, by periodic monitoring of one or more serum lipid levels, *e.g.*, triglyceride levels. Comparisons of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of an iRNA or pharmaceutical composition thereof, "effective against" a disorder of lipid metabolism indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as an improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating disorder of lipid metabolisms and the related causes.

A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment.

5 Efficacy for a given iRNA drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art.

The invention further provides methods for the use of a iRNA agent or a pharmaceutical composition of the invention, *e.g.*, for treating a subject that would benefit from reduction and/or inhibition of CIDEB expression or CIDEB, *e.g.*, a subject having a CIDEB-associated disease disorder, or condition, in
10 combination with other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders. In some embodiments, the invention provides methods for the use of a iRNA agent or a pharmaceutical composition of the invention and an iRNA agent targeting PNPLA3, *e.g.*, for treating a subject that would benefit from reduction and/or inhibition of CIDEB expression and PNPLA3 expression,
15 *e.g.*, a subject having a CIDEB-associated disease disorder, or condition (*e.g.*, chronic inflammatory disease), in combination with other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders. For example, in certain embodiments, an iRNA agent or pharmaceutical composition of the invention is administered in combination with, *e.g.*, pyridoxine, an ACE inhibitor
20 (angiotensin converting enzyme inhibitors), *e.g.*, benazepril agents to decrease blood pressure, *e.g.*, diuretics, beta-blockers, ACE inhibitors, angiotensin II receptor blockers, calcium channel blockers, alpha blockers, alpha-2 receptor antagonists, combined alpha- and beta-blockers, central agonists, peripheral adrenergic inhibitors, and blood vessel dilators; or agents to decrease cholesterol, *e.g.*, statins, selective cholesterol absorption inhibitors, resins; lipid lowering therapies; insulin sensitizers, such as the PPAR γ agonist
25 pioglitazone; glp-1r agonists, such as liraglutide; vitamin E; SGLT2 inhibitors; or DPPIV inhibitors; or a combination of any of the foregoing. In one embodiment, an iRNA agent or pharmaceutical composition of the invention is administered in combination with an agent that inhibits the expression and/or activity of a transmembrane 6 superfamily member 2 (TM6SF2) gene, *e.g.*, an RNAi agent that inhibits the expression of a TM6SF2 gene.

30 The iRNA agent and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, *e.g.*, subcutaneously, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

VIII. Kits

The present invention also provides kits for performing any of the methods of the invention. Such kits include one or more RNAi agent(s) and instructions for use, *e.g.*, instructions for inhibiting expression of a CIDEB in a cell by contacting the cell with an RNAi agent or pharmaceutical composition of the invention in an amount effective to inhibit expression of the CIDEB. The kits may optionally further comprise means for contacting the cell with the RNAi agent (*e.g.*, an injection device), or means for measuring the inhibition of CIDEB (*e.g.*, means for measuring the inhibition of CIDEB mRNA and/or CIDEB protein). Such means for measuring the inhibition of CIDEB may comprise a means for obtaining a sample from a subject, such as, *e.g.*, a plasma sample. The kits of the invention may optionally further comprise means for administering the RNAi agent(s) to a subject or means for determining the therapeutically effective or prophylactically effective amount.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the iRNAs and methods featured in the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

Example 1. CIDEB iRNA Design, Synthesis, and Selection

Nucleic acid sequences provided herein are represented using standard nomenclature. *See* the abbreviations of Table 2. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds. The abbreviations are understood to omit the 3'-phosphate (*i.e.* they are 3'-OH) when placed at the 3'-terminal position of an oligonucleotide.

TABLE 2: Abbreviations of nucleotide monomers used in nucleic acid sequence representation.

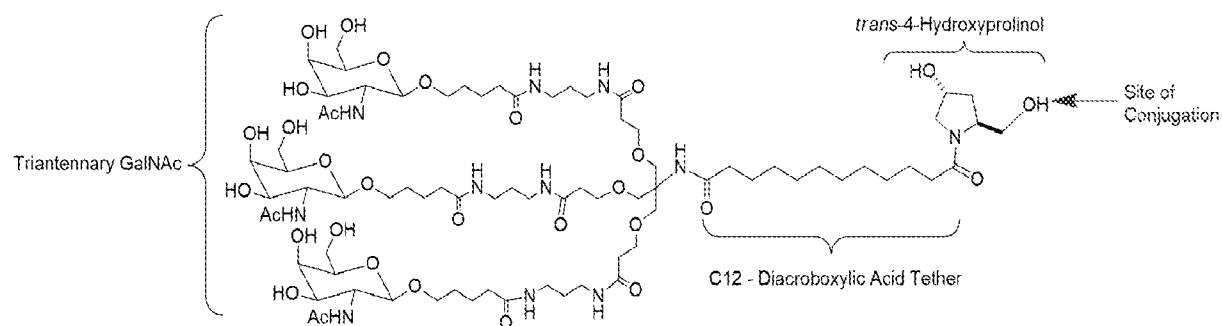
Abbreviation	Nucleotide(s)
A	Adenosine-3'-phosphate
Ab	beta-L-adenosine-3'-phosphate
Abs	beta-L-adenosine-3'-phosphorothioate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate

Abbreviation	Nucleotide(s)
As	adenosine-3'-phosphorothioate
(A2p)	adenosine-2'-phosphate
(A2ps)	adenosine-2'-phosphorothioate
C	cytidine-3'-phosphate
Cb	beta-L-cytidine-3'-phosphate
Cbs	beta-L-cytidine-3'-phosphorothioate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
(C2p)	cytidine-2'-phosphate
(C2ps)	cytidine-2'-phosphorothioate
G	guanosine-3'-phosphate
Gb	beta-L-guanosine-3'-phosphate
Gbs	beta-L-guanosine-3'-phosphorothioate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
(G2p)	guanosine-2'-phosphate
(G2ps)	guanosine-2'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
U	Uridine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine -3'-phosphorothioate
Us	uridine -3'-phosphorothioate
(U2p)	uridine-2'-phosphate
(U2ps)	uridine-2'-phosphorothioate
N	any nucleotide (G, A, C, T or U)
a	2'-O-methyladenosine-3'-phosphate
as	2'-O-methyladenosine-3'-phosphorothioate
c	2'-O-methylcytidine-3'-phosphate
cs	2'-O-methylcytidine-3'-phosphorothioate

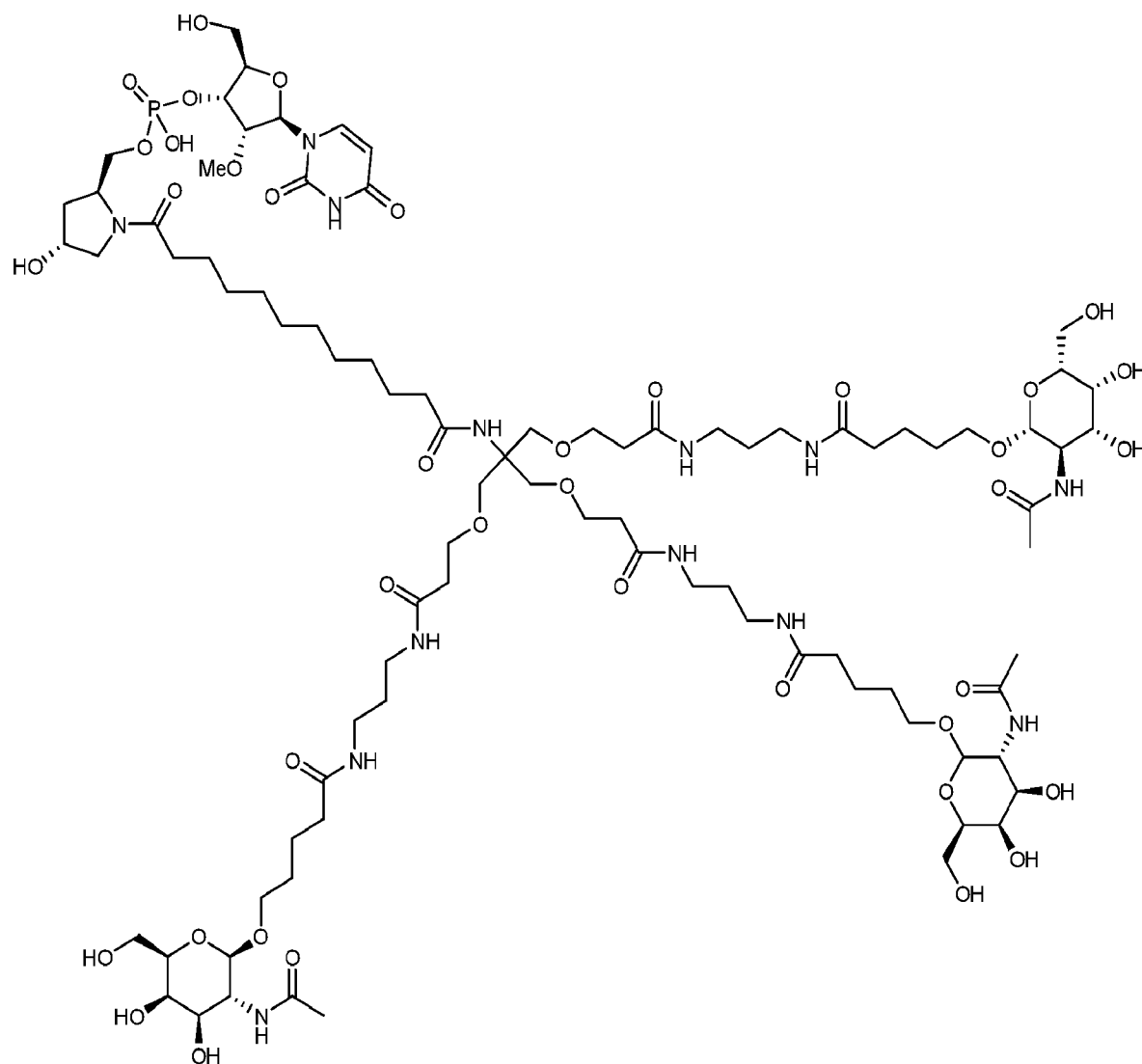
Abbreviation	Nucleotide(s)
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'-phosphorothioate
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
s	phosphorothioate linkage
L96 ¹	N-[tris(GalNAc-alkyl)-amidodecanoyl]-4-hydroxyprolinol; or (2S,4R)-1-[29-[[2-(acetylamino)-2-deoxy-β-D-galactopyranosyl]oxy]-14,14-bis[[3-[[3-[[5-[[2-(acetylamino)-2-deoxy-β-D-galactopyranosyl]oxy]-1-oxopentyl] amino]propyl]amino]-3-oxopropoxy]methyl]-1,12,19,25-tetraoxo-16-oxa-13,20,24-triazanonacos-1-yl]-4-hydroxy-2-hydroxymethylpyrrolidine
uL96 ²	2'-O-methyluridine-3'-phosphate ((2S,4R)-1-[29-[[2-(acetylamino)-2-deoxy-β-D-galactopyranosyl]oxy]-14,14-bis[[3-[[3-[[5-[[2-(acetylamino)-2-deoxy-β-D-galactopyranosyl]oxy]-1-oxopentyl]amino]propyl]amino]-3-oxopropoxy]methyl]-1,12,19,25-tetraoxo-16-oxa-13,20,24-triazanonacos-1-yl]-4-hydroxy-2-pyrrolidinyl)methyl ester
P	Phosphate
VP	Vinyl-phosphate
dA	2'-deoxyadenosine-3'-phosphate
dAs	2'-deoxyadenosine-3'-phosphorothioate
dC	2'-deoxycytidine-3'-phosphate
dCs	2'-deoxycytidine-3'-phosphorothioate
dG	2'-deoxyguanosine-3'-phosphate
dGs	2'-deoxyguanosine-3'-phosphorothioate
dT	2'-deoxythymidine-3'-phosphate
dTs	2'-deoxythymidine-3'-phosphorothioate
dU	2'-deoxyuridine
dUs	2'-deoxyuridine-3'-phosphorothioate
Y34	2-hydroxymethyl-tetrahydrofuran-4-methoxy-3-phosphate (abasic 2'-OMe furanose)
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofuran-5-phosphate)
(Agn)	Adenosine-glycol nucleic acid (GNA)
(Cgn)	Cytidine-glycol nucleic acid (GNA)

Abbreviation	Nucleotide(s)
(Ggn)	Guanosine-glycol nucleic acid (GNA)
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
(Aam)	2'-O-(N-methylacetamide)adenosine-3'-phosphate
(Aams)	2'-O-(N-methylacetamide)adenosine-3'-phosphorothioate
(Gam)	2'-O-(N-methylacetamide)guanosine-3'-phosphate
(Gams)	2'-O-(N-methylacetamide)guanosine-3'-phosphorothioate
(Tam)	2'-O-(N-methylacetamide)thymidine-3'-phosphate
(Tams)	2'-O-(N-methylacetamide)thymidine-3'-phosphorothioate
(Aeo)	2'-O-methoxyethyladenosine-3'-phosphate
(Aeos)	2'-O-methoxyethyladenosine-3'-phosphorothioate
(Geo)	2'-O-methoxyethylguanosine-3'-phosphate
(Geos)	2'-O-methoxyethylguanosine-3'-phosphorothioate
(Teo)	2'-O-methoxyethyl-5-methyluridine-3'-phosphate
(Teos)	2'-O-methoxyethyl-5-methyluridine-3'-phosphorothioate
(m5Ceo)	2'-O-methoxyethyl-5-methylcytidine-3'-phosphate
(m5Ceos)	2'-O-methoxyethyl-5-methylcytidine-3'-phosphorothioate
(A3m)	3'-O-methyladenosine-2'-phosphate
(A3mx)	3'-O-methyl-xylofuranosyladenosine-2'-phosphate
(G3m)	3'-O-methylguanosine-2'-phosphate
(G3mx)	3'-O-methyl-xylofuranosylguanosine-2'-phosphate
(C3m)	3'-O-methylcytidine-2'-phosphate
(C3mx)	3'-O-methyl-xylofuranosylcytidine-2'-phosphate
(U3m)	3'-O-methyluridine-2'-phosphate
(U3mx)	3'-O-methyl-xylofuranosyluridine-2'-phosphate
(m5Cam)	2'-O-(N-methylacetamide)-5-methylcytidine-3'-phosphate
(m5Cams)	2'-O-(N-methylacetamide)-5-methylcytidine-3'-phosphorothioate
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(Chds)	2'-O-hexadecyl-cytidine-3'-phosphorothioate
(Uhd)	2'-O-hexadecyl-uridine-3'-phosphate
(Uhds)	2'-O-hexadecyl-uridine-3'-phosphorothioate
(pshe)	Hydroxyethylphosphorothioate

¹The chemical structure of L96 is as follows:



²The chemical structure of uL96 is as follows:



5 Experimental Methods

This Example describes methods for the design, synthesis, and selection of CIDEB iRNA agents.

Bioinformatics

Source of reagents

Where the source of a reagent is not specifically given herein, such reagent can be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

Transcripts

5 A set of siRNAs targeting the human Cell death-inducing DFFA-like effector B (CIDEB; human NCBI refseqID NM_001393338.1; NCBI GeneID: 27141), were designed using custom R and Python scripts. The human NM_001393338.1 REFSEQ mRNA has a length of 2482 bases.

siRNA Synthesis

10 siRNAs were synthesized and annealed using routine methods known in the art.
Briefly, siRNA sequences were synthesized at 1 μ mol scale on a Mermade 192 synthesizer (BioAutomation) using the solid support mediated phosphoramidite chemistry. The solid support was controlled pore glass (500 Å) loaded with custom GalNAc ligand or universal solid support (AM biochemical). Ancillary synthesis reagents, 2'-F and 2'-O-Methyl RNA and deoxy phosphoramidites were
15 obtained from Thermo-Fisher (Milwaukee, WI) and Hongene (China). 2'-F 2'-O-Methyl, GNA (glycol nucleic acids), 5' phosphate and other modifications were introduced using the corresponding phosphoramidites. Synthesis of 3' GalNAc conjugated single strands was performed on a GalNAc modified CPG support. Custom CPG universal solid support was used for the synthesis of antisense single strands. Coupling time for all phosphoramidites (100 mM in acetonitrile) was 5 min employing 5-Ethylthio-1H-tetrazole (ETT) as
20 activator (0.6 M in acetonitrile). Phosphorothioate linkages were generated using a 50 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (1:1 v/v). Oxidation time was 3 minutes. All sequences were synthesized with final removal of the DMT group ("DMT off").

Upon completion of the solid phase synthesis, oligoribonucleotides were cleaved from the solid
25 support and deprotected in sealed 96 deep well plates using 200 μ L Aqueous Methylamine reagents at 60°C for 20 minutes. For sequences containing 2' ribo residues (2'-OH) that are protected with a tert-butyl dimethyl silyl (TBDMS) group, a second step deprotection was performed using TEA.3HF (triethylamine trihydro fluoride) reagent. To the methylamine deprotection solution, 200 μ L of dimethyl sulfoxide (DMSO) and 300 μ L TEA.3HF reagent was added and the solution was incubated for additional 20 min at 60°C. At the end of
30 cleavage and deprotection step, the synthesis plate was allowed to come to room temperature and was precipitated by addition of 1 mL of acetone: ethanol mixture (9:1). The plates were cooled at -80°C for 2 hrs, supernatant decanted carefully with the aid of a multi-channel pipette. The oligonucleotide pellet was re-suspended in 20 mM NaOAc buffer and were desalted using a 5 mL HiTrap size exclusion column (GE Healthcare) on an AKTA Purifier System equipped with an A905 autosampler and a Frac 950 fraction
35 collector. Desalted samples were collected in 96-well plates. Samples from each sequence were analyzed by

LC-MS to confirm the identity, UV (260 nm) for quantification and a selected set of samples by IEX chromatography to determine purity.

Annealing of single strands was performed on a Tecan liquid handling robot. Equimolar mixture of sense and antisense single strands were combined and annealed in 96 well plates. After combining the complementary single strands, the 96-well plate was sealed tightly and heated in an oven at 100°C for 10 minutes and allowed to come slowly to room temperature over a period 2-3 hours. The concentration of each duplex was normalized to 10µM in 1X PBS and then submitted for *in vitro* screening assays.

In some instances, a duplex (dsRNA) was synthesized more than once. Different batches are labeled with different extensions. For example, AD-1686813.1 and AD-1686813.2 are different batches of the same duplex. Duplexes having the same ID but without an extension, or with different extensions, have the same nucleotide sequences of the sense strand and antisense strand, e.g., AD-1686813, AD-1686813.1, and AD-1686813.2 have the same nucleotide sequences of the sense strand and antisense strand.

A detailed list of the unmodified nucleotide sequences of the sense strand and antisense strand sequences is shown in Tables 3 and 5.

A detailed list of the modified nucleotide sequences of the sense strand and antisense strand sequences is shown in Tables 4 and 6.

TABLE 3. Unmodified Sense and Antisense Strand Sequences of Human CIDEA dsRNA Agents

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM 001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM 001393338.1
AD-1685156	CUGCAGAAGGU UGACUGCGUU	7	31-51	AACGCAGUCAAC CUUCUGCAGGC	142	29-51
AD-1685173	AGCCGAAGGCA AGCACGAUGU	8	69-89	ACAUCGUGCUUG CCUUCGGCUUG	143	67-89
AD-1685198	CCAGCGUCACG CUGUAGCAGU	9	156-176	ACUGCUACAGCG UGACGCUGGCA	144	154-176
AD-1685207	CGCUGUAGCAG CCGAGCAUCU	10	165-185	AGAUGCUCGGCU GCUACAGCGUG	145	163-185
AD-1685217	GCCGAGCAUCA GCCCCGAAAGU	11	175-195	ACUUUCGGGCUG AUGCUCGGCUG	146	173-195
AD-1685228	GCCCCGAAAGGA AGCACGAAAU	12	186-206	AUUUCGUGCUUC CUUUCGGGCUG	147	184-206
AD-1685240	GCACGAAAGCG GUCAGAGUCU	13	198-218	AGACUCUGACCG CUUUCGUGCUU	148	196-218
AD-1685250	GGUCAGAGUCU CCAGGCUCAU	14	208-228	AUGAGCCUGGAG ACUCUGACCGC	149	206-228
AD-1685271	GCACAGCUGGC AUACGCGGUU	15	259-279	AACCGCGUAUGC CAGCUGUGCCA	150	257-279
AD-1685284	ACGCGGUCCCU CCACAGGUGU	16	272-292	ACACCUGUGGAG GGACCGCGUAU	151	270-292
AD-1685366	GCUGGCGUACA UGCUGAGCGU	17	448-468	ACGCUCAGCAUG UACGCCAGCGU	152	446-468
AD-1685379	CUGAGCGCGCA CACGUAGUAU	18	461-481	AUACUACGUGUG CGCGCUCAGCA	153	459-481

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001393338.1
AD-1685388	CACACGUAGUA CACCGCCUUU	19	470-490	AAAGGCGGUGUA CUACGUGUGCG	154	468-490
AD-1685406	CCGGGUCAGGA AGGCCACAAU	20	520-540	AUUGUGGCCUUC CUGACCCCGCA	155	518-540
AD-1685418	GGCCACAAAGA GCGGCGUGAU	21	532-552	AUCACGCCGCUC UUUGUGGCCUU	156	530-552
AD-1685469	CCACACCACGA AGCCGUUGCU	22	643-663	AGCAACGGCUUC GUGGUGUGGAG	157	641-663
AD-1685491	AGCAGGAAGGC UGUGCCUGUU	23	689-709	AACAGGCACAGC CUUCCUGCUGC	158	687-709
AD-1685503	CCUGUGGCCCCG CGAAGUCUUU	24	704-724	AAAGACUUCGCG GGCCACAGGCA	159	702-724
AD-1685512	CGCGAAGUCUU CCAGCUCAGU	25	713-733	ACUGAGCUGGAA GACUUCGCGGG	160	711-733
AD-1685528	UCAGCAGUGUC UCGUUCCCUU	26	729-749	AAGGGAACGAGA CACUGCUGAGC	161	727-749
AD-1685538	GCAGACCGACA UCCUUCUGGU	27	760-780	ACCAGAAGGAUG UCGGUCUGCUA	162	758-780
AD-1685549	UCCUUCUGGGC CUACAGCCUU	28	771-791	AAGGCUGUAGGC CCAGAAGGAUG	163	769-791
AD-1685561	UACAGCCUGCC UCUUUUCUGU	29	783-803	ACAGAAAAGAGG CAGGCUGUAGG	164	781-803
AD-1685570	CCUCUUUUCUG CCUGGGAGUU	30	792-812	AACUCCCAGGCA GAAAAGAGGCA	165	790-812
AD-1685587	AGUCCUGACUU CCACGAGGAU	31	809-829	AUCCUCGUGGAA GUCAGGACUCC	166	807-829
AD-1685599	CUCAAACACAA CUCCUUCUUU	32	841-861	AAAGAAGGAGUU GUGUUUGAGGU	167	839-861
AD-1685610	CUCCUUCUUGG AACCCAGAUU	33	852-872	AAUCUGGGUUC AAGAAGGAGUU	168	850-872
AD-1685614	UGCUCCCAGUC AGUUGACCUU	34	876-896	AAGGUCAACUGA CUGGGAGCAGG	169	874-896
AD-1685627	CCUCCUCCCCA GAGCUCAGUU	35	909-929	AACUGAGCUCUG GGAAGGAGGCC	170	907-929
AD-1685637	AGAGCUCAGUG GACACAGAAU	36	919-939	AUUCUGUGUCCA CUGAGCUCUGG	171	917-939
AD-1685648	ACCCUACAAGG AUCCUUGGCU	37	960-980	AGCCAAGGAUCC UUGUAGGGUCC	172	958-980
AD-1685664	UGGCAGGAAAG CAGGGAUUGU	38	976-996	ACAAUCCUGCU UUCUGCCAAG	173	974-996
AD-1685673	AGCAGGGAUUG UGUUCAUUUU	39	985-1005	AAAAUGAACACA AUCCUGCUUU	174	983-1005
AD-1685689	AUUUGAGGGUU UCACUGUCAU	40	1001-1021	AUGACAGUGAAA CCCUCAAUGA	175	999-1021
AD-1685699	UUCACUGUCAG UGAGAGUCUU	41	1011-1031	AAGACUCUCACU GACAGUGAAAC	176	1009-1031
AD-1685708	AGUGAGAGUCU CAGCUUCCA	42	1020-1040	AUGGAAGCUGAG ACUCUCACUGA	177	1018-1040

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001393338.1
AD-1685717	CUCAGCUUCCA UGCAACUGUU	43	1029-1049	AACAGUUGCAUG GAAGCUGAGAC	178	1027-1049
AD-1685726	CAUGCAACUGU CCAUCACGGU	44	1038-1058	ACCGUGAUGGAC AGUUGCAUGGA	179	1036-1058
AD-1685735	GUCCAUCACGG CUGCAACUGU	45	1047-1067	ACAGUUGCAGCC GUGAUGGACAG	180	1045-1067
AD-1685744	GGCUGCAACUG AAAUCAGAGU	46	1056-1076	ACUCUGAUUUCA GUUGCAGCCGU	181	1054-1076
AD-1685770	CACAGCGCACC AGAAGCUAAU	47	1082-1102	AUUAGCUUCUGG UGCGCUGUGUC	182	1080-1102
AD-1685779	CCAGAAGCUAA AGUCUUGAUU	48	1091-1111	AAUCAAGACUUU AGCUUCUGGUG	183	1089-1111
AD-1685788	AAAGUCUUGAU GCCAUCAAAU	49	1100-1120	AUUUGAUGGCAU CAAGACUUUAG	184	1098-1120
AD-1685798	UGCCAUCAAAG GACAUCCCUU	50	1110-1130	AAGGGAUGUCCU UUGAUGGCAUC	185	1108-1130
AD-1685810	UCUCUGUCACG UCCACUAAAU	51	1142-1162	AAUUAGUGGACG UGACAGAGAUG	186	1140-1162
AD-1685826	UAAUCGGCAAA AGGAGAAAAU	52	1158-1178	AUUUUCUCCUUU UGCCGAUUAGU	187	1156-1178
AD-1685838	AAGUGAGAGAA GAUGACCUAU	53	1176-1196	AUAGGUCAUCUU CUCUCACUUUU	188	1174-1196
AD-1685847	AAGAUGACCUA AGUGUGACUU	54	1185-1205	AAGUCACACUUA GGUCAUCUUCU	189	1183-1205
AD-1685856	UAAGUGUGACU GCAGCAGGCU	55	1194-1214	AGCCUGCUGCAG UCACACUUAGG	190	1192-1214
AD-1685869	AGCAGGCAGCU CUGGAAAAAU	56	1207-1227	AAUUUCCAGAG CUGCCUGCUGC	191	1205-1227
AD-1685878	CUCUGGAAAAU GAAGCCAGAU	57	1216-1236	AUCUGGCUUCAU UUUCCAGAGCU	192	1214-1236
AD-1685889	GAAGCCAGAGC AGUGAGCCAU	58	1227-1247	AUGGCUCACUGC UCUGGCUUCAU	193	1225-1247
AD-1685898	CCGACCAAGGA GGAAGGAAAU	59	1256-1276	AUUUCCUCCUC CUUGGUCGGAG	194	1254-1276
AD-1685902	GAGGAAGGAAA GAGCAGAUCU	60	1265-1285	AGAUCUGCUCUU UCCUUCCUCCU	195	1263-1285
AD-1685912	AGAGCAGAUCC CAGGUUUGUU	61	1275-1295	AACAAACCUGGG AUCUGCUCUUU	196	1273-1295
AD-1685921	CCCAGGUUUGU AACAGAAAAU	62	1284-1304	AUUUUCUGUUAU AAACCUGGGAU	197	1282-1304
AD-1685931	UAACAGAAAAC ACCACUAAAU	63	1294-1314	AUUUAGUGGUGU UUUCUGUUACA	198	1292-1314
AD-1685935	CAGCACAGGAG AGAACCACCU	64	1318-1338	AGGUGGUUCUCU CCUGUGCUGGG	199	1316-1338
AD-1685956	AGCCCAGAAGU UCCAGGGAAU	65	1339-1359	AUUCCUGGAAC UUCUGGGCUGG	200	1337-1359
AD-1685969	CAGGGAAGGAA CUCUCCGGUU	66	1352-1372	AACCGGAGAGUU CCUUCCUGGA	201	1350-1372

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001393338.1
AD-1685983	UCCGGUCCACC AUGGAGUACU	67	1366-1386	AGUACUCCAUGG UGGACCGGAGA	202	1364-1386
AD-1685994	AUGGAGUACCU CUCAGCUCUU	68	1377-1397	AAGAGCUGAGAG GUACUCCAUGG	203	1375-1397
AD-1686007	UUACUCAGGUC AGUAUCUAAU	69	1410-1430	AUUAGAUACUGA CCUGAGUAAAGU	204	1408-1430
AD-1686016	UCAGUAUCUAA UAUAAGCUCU	70	1419-1439	AGAGCUUAUAUU AGAUACUGACC	205	1417-1439
AD-1686028	AUAAGCUCGGA GUUUGGACGU	71	1431-1451	ACGUCCAAACUC CGAGCUUAUAU	206	1429-1451
AD-1686053	UCUGGACCUC GCUCCACCAU	72	1456-1476	AUGGUGGAGCUG AGGUCCAGACC	207	1454-1476
AD-1686057	AGCGACCUUUC CGUGUCUGUU	73	1480-1500	AACAGACACGGA AAGGUCGCUGG	208	1478-1500
AD-1686066	UCCGUGUCUGU GAUCACAAGU	74	1489-1509	ACUUGUGAUCAC AGACACGGAAA	209	1487-1509
AD-1686075	GUGAUCACAAG CGGACCAUCU	75	1498-1518	AGAUGGUCCGCU UGUGAUCACAG	210	1496-1518
AD-1686086	CGGACCAUCCG GAAAGGCCUU	76	1509-1529	AAGGCCUUUCCG GAUGGUCCGCU	211	1507-1529
AD-1686098	AAAGGCCUGAC AGCUGCCACU	77	1521-1541	AGUGGCAGCUGU CAGGCCUUUCC	212	1519-1541
AD-1686116	CCAGGAGCUGC UAGCCAAAGU	78	1544-1564	ACUUUGGCUAGC AGCUCCUGGCG	213	1542-1564
AD-1686126	CUAGCCAAAGC AUUGGAGACU	79	1554-1574	AGUCUCCAAUGC UUUGGCUAGCA	214	1552-1574
AD-1686136	CAUUGGAGACC CUACUGCUGU	80	1564-1584	ACAGCAGUAGGG UCUCCAAUGCU	215	1562-1584
AD-1686147	CUACUGCUGAA UGGAGUGCUU	81	1575-1595	AAGCACUCCAUA CAGCAGUAGGG	216	1573-1595
AD-1686159	GGAGUGCUAAC CCUGGUGCUU	82	1587-1607	AAGCACCAGGGU UAGCACUCCA	217	1585-1607
AD-1686171	CUGGUGCUGAG GGAGGAUGGU	83	1599-1619	ACCAUCCUCCUC UAGCACCAGGG	218	1597-1619
AD-1686181	AGGAGGAUGGA ACUGCAGUGU	84	1609-1629	ACACUGCAGUUC CAUCCUCCUCU	219	1607-1629
AD-1686197	AGUGGACAGUG AGGACUUCUU	85	1625-1645	AAGAAGUCCUCA CUGUCCACUGC	220	1623-1645
AD-1686207	GAGGACUUCUU CCAGCUGCUU	86	1635-1655	AAGCAGCUGGAA GAAGUCCUCAC	221	1633-1655
AD-1686216	UCCAGCUGCU GGAGGAUGAU	87	1644-1664	AUCAUCCUCCAG CAGCUGGAAGA	222	1642-1664
AD-1686225	CUGGAGGAUGA CACGUGCCUU	88	1653-1673	AAGGCACGUGUC AUCCUCCAGCA	223	1651-1673
AD-1686242	CCUGAUGGUGU UGCAGUCUGU	89	1670-1690	ACAGACUGCAAC ACCAUCAGGCA	224	1668-1690
AD-1686251	GUUGCAGUCUG GUCAGAGCUU	90	1679-1699	AAGCUCUGACCA GACUGCAACAC	225	1677-1699

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001393338.1
AD-1686265	AGAGCUGGAGC CCUACAAGGU	91	1693-1713	ACCUUGUAGGGC UCCAGCUCUGA	226	1691-1713
AD-1686274	GCCCUACAAGG AGUGGAGUGU	92	1702-1722	ACACUCCACUCC UUGUAGGGCUC	227	1700-1722
AD-1686286	GUGGAGUGCUG UCAUAUGGCU	93	1714-1734	AGCCAU AUGACA GCACUCCACUC	228	1712-1734
AD-1686319	CAAGCACAGCA AGGACAUCGU	94	1751-1771	ACGAUGUCCUUG CUGUGCUUGGG	229	1749-1771
AD-1686334	CAUCGCCCCGAU UCACCUUUGU	95	1766-1786	ACAAAGGUGAAU CGGGCGAUGUC	230	1764-1786
AD-1686343	AUUCACCUUUG ACGUGUACAU	96	1775-1795	AUGUACACGUCA AAGGUGAAUCG	231	1773-1795
AD-1686354	ACGUGUACAAG CAAAACCCUU	97	1786-1806	AAGGGUUUUGCU UGUACACGUCA	232	1784-1806
AD-1686366	AAAACCCUCGA GACCUCUUUU	98	1798-1818	AAAAGAGGUCUC GAGGGUUUUGC	233	1796-1818
AD-1686377	GACCUCUUUGG CAGCCUGAAU	99	1809-1829	AUUCAGGCUGCC AAAGAGGUCUC	234	1807-1829
AD-1686394	GAAUGUCAAAAG CCACAUUCUU	100	1826-1846	AAGAAUGUGGCU UUGACAUUCAG	235	1824-1846
AD-1686403	AGCCACAUUCU ACGGGCUCUU	101	1835-1855	AAGAGCCCGUAG AAUGUGGCUUU	236	1833-1855
AD-1686417	GGCUCUACUCU AUGAGUUGUU	102	1849-1869	AACAACUCAUAG AGUAGAGCCCG	237	1847-1869
AD-1686426	CUAUGAGUUGU GACUUUCAAU	103	1858-1878	AUUGAAAGUCAC AACUCAUAGAG	238	1856-1878
AD-1686435	GUGACUUUCAA GGACUUGGCU	104	1867-1887	AGCCAAGUCCUU GAAAGUCACAA	239	1865-1887
AD-1686451	UGGCCCAAAGA AAGUACUCAU	105	1883-1903	AUGAGUACUUUC UUUGGGCCAAG	240	1881-1903
AD-1686466	ACUCAGGGAGC UCCUUCGUUU	106	1898-1918	AAACGAAGGAGC UCCCUGAGUAC	241	1896-1918
AD-1686475	GCUCCUUCGUU GGACCUCAU	107	1907-1927	AUGGAGGUCCAA CGAAGGAGCUC	242	1905-1927
AD-1686491	UCCACACUGCU GCAAGGCCUU	108	1923-1943	AAGGCCUUGCAG CAGUGUGGAGG	243	1921-1943
AD-1686508	CCUGGGCCAUA UGUUGCUGGU	109	1940-1960	ACCAGCAACAUA UGGCCAGGCC	244	1938-1960
AD-1686518	AUGUUGCUGGG AAUUUCCUCU	110	1950-1970	AGAGGAAAUUCC CAGCAACAUAU	245	1948-1970
AD-1686531	UUUCCUCCACC CUUCGUCAUU	111	1963-1983	AAUGACGAAGGG UGGAGGAAAUU	246	1961-1983
AD-1686540	CCCUUCGUCAU GCAGUGGAGU	112	1972-1992	ACUCCACUGCAU GACGAAGGGUG	247	1970-1992
AD-1686543	CUGAGCAGUGG CAGCAGAAGU	113	1996-2016	ACUUCUGCUGCC ACUGCUCAGCC	248	1994-2016
AD-1686556	CAGAAGGGCCG CCUCCAUAUCU	114	2010-2030	AGAAUGGAGGCG GCCCCUUCUGCU	249	2008-2030

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001393338.1
AD-1686565	CGCCUCCAUUC CUACUAAGGU	115	2019-2039	ACCUUAGUAGGA AUGGAGGCGGC	250	2017-2039
AD-1686566	CAGAAUCAUUC CAACCGACCU	116	2058-2078	AGGUCGGUUGGA AUGAUUCUGGG	251	2056-2078
AD-1686576	CCAACCGACCC ACUGCAAAGU	117	2068-2088	ACUUUGCAGUGG GUCGGUUGGAA	252	2066-2088
AD-1686585	CCACUGCAAAG ACUAUGACAU	118	2077-2097	AUGUCAUAGUCU UUGCAGUGGGU	253	2075-2097
AD-1686597	CUAUGACAGCA UCAAUUUUCU	119	2089-2109	AGAAAUUUGAUG CUGUCAUAGUC	254	2087-2109
AD-1686606	CAUCAAAUUUC AGGACCUGCU	120	2098-2118	AGCAGGUCCUGA AAUUUGAUGCU	255	2096-2118
AD-1686616	CAGGACCUGCA GACAGUACAU	121	2108-2128	AUGUACUGUCUG CAGGUCCUGAA	256	2106-2128
AD-1686626	AGACAGUACAG GCUAGAUAUU	122	2118-2138	AUUUUCUAGCCU GUACUGUCUGC	257	2116-2138
AD-1686639	UAGAUAAACCA CCCAAUUUCU	123	2131-2151	AGAAAUUGGGUG GGUUAUCUAGC	258	2129-2151
AD-1686649	AGAACCUUUCA GCAUAACGCU	124	2178-2198	AGCGUUAUGCUG AAAGGUUCUGU	259	2176-2198
AD-1686658	CAGCAUAACGC CUCACAUCCU	125	2187-2207	AGGAUGUGAGGC GUUAUGCUGAA	260	2185-2207
AD-1686669	CUCACAUCCCA AGUCUAUACU	126	2198-2218	AGUAUAGACUUG GGAUGUGAGGC	261	2196-2218
AD-1686680	AGUCUAUACCC UUACCUGAAU	127	2209-2229	AUUCAGGUAAGG GUAUAGACUUG	262	2207-2229
AD-1686693	ACCUGAAGAAU GCUGUUCUUU	128	2222-2242	AAAGAACAGCAU UCUUCAGGUAA	263	2220-2242
AD-1686709	UCUUUCCUAGC CACC UUUCUU	129	2238-2258	AAGAAAGGUGGC UAGGAAAGAAC	264	2236-2258
AD-1686720	CACC UUUCUGG CCUCCACUU	130	2249-2269	AAGUGGGAGGCC AGAAAGGUGGC	265	2247-2269
AD-1686729	GGCCUCCACU UGCCUGAAU	131	2258-2278	AUUCAGGGCAAG UGGGAGGCCAG	266	2256-2278
AD-1686738	CUUGCCCUGAA AGGCCAAGAU	132	2267-2287	AUCUUGGCCUUU CAGGGCAAGUG	267	2265-2287
AD-1686747	AAAGGCCAAGA UCAAGAUGUU	133	2276-2296	AACAUCUUGAUC UUGGCCUUUCA	268	2274-2296
AD-1686751	AGGCAUCUUGA UCCAGCCUU	134	2301-2321	AAGGCUGGGAUC AAGAUGCCUGG	269	2299-2321
AD-1686761	AUCCAGCCUG ACUGCUGCUU	135	2311-2331	AAGCAGCAGUCA GGCUGGGAUCA	270	2309-2331
AD-1686770	UGACUGCUGCU ACAUCUAAUU	136	2320-2340	AAUUAGAUGUAG CAGCAGUCAGG	271	2318-2340
AD-1686781	GCCUCCUGUCC CUAAACUCCU	137	2351-2371	AGGAGUUUAGGG ACAGGAGGCAU	272	2349-2371
AD-1686783	AGCAUACUGAU GACAGCCCUU	138	2373-2393	AAGGGCUGUCAU CAGUAUGCUGG	273	2371-2393

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001393338.1
AD-1686794	GACAGCCCUCU CUGACUUUAU	139	2384-2404	AUAAAGUCAGAG AGGGCUGUCAU	274	2382-2404
AD-1686803	CUCUGACUUUA CCUUGAGAUU	140	2393-2413	AAUCUCAAGGUA AAGUCAGAGAG	275	2391-2413
AD-1686813	ACCUUGAGAUC UGUCUUCAU	141	2403-2423	AAUGAAGACAGA UCUCAAGGUAA	276	2401-2423

TABLE 4. Modified Sense and Antisense Strand Sequences of Human CIDEB dsRNA Agents

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1685156	csusgcagAfaGfGfUfuga cugcgguL96	277	asAfscgcAfgUfCfaa ccUfuCfugcagsgsc	412	GCCUGCAGAAGGU UGACUGCGUG	547
AD-1685173	asgscgaAfgGfCfAfac acgauguL96	278	asCfsaucGfuGfCfu gcCfuUfcggcususg	413	CAAGCCGAAGGCA AGCACGAUGG	548
AD-1685198	cscsagcgUfcAfCfGfcg uagcaguL96	279	asCfsugcUfaCfAfgc guGfaCfcgugscsa	414	UGCCAGCGUCACG CUGUAGCAGC	549
AD-1685207	csgscuguAfgCfAfGfccg agcaucuL96	280	asGfsaugCfuCfGfgc ugCfuAfcagcgsusg	415	CACGCUGUAGCAG CCGAGCAUCA	550
AD-1685217	gscscgagCfaUfCfAfgcc cgaaaguL96	281	asCfsuuuUfcGfGfcu gaUfgCfucggcsusg	416	CAGCCGAGCAUCA GCCCCGAAAGG	551
AD-1685228	gscscgaAfaGfGfAfac acgaaauL96	282	asUfsuucGfuGfCfu ccUfuUfcggcsusg	417	CAGCCGAAAGGA AGCACGAAAG	552
AD-1685240	gscsacgaAfaGfCfGfguc agagucuL96	283	asGfsacuCfuGfAfc gcUfuUfcgugcsusu	418	AAGCACGAAAGCG GUCAGAGUCU	553
AD-1685250	gsgsucagAfgUfCfUfcc ggcucauL96	284	asUfsgagCfcUfGfga gaCfuCfugaccsgsc	419	GCGGUCAGAGUCU CCAGGCUCAG	554
AD-1685271	gscsacagCfuGfGfCfaa cgcgguL96	285	asAfscgcCfuUfAfu ccAfgCfugugscsa	420	UGGCACAGCUGGC AUACGCGGUC	555
AD-1685284	ascsgeggUfcCfCfUfcc cagguguL96	286	asCfsaccUfgUfGfga ggGfaCfcgugcsasu	421	AUACGCGGUCCU CCACAGGUGG	556
AD-1685366	gscsuggcGfuAfCfAfu cugagcguL96	287	asCfsgcuCfaGfCfa guAfcGfccagcsusu	422	ACGCUGGCGUACA UGCUGAGCGC	557
AD-1685379	csusgagGfcGfCfAfcac guaguauL96	288	asUfsacuAfcGfUfgu gcGfcGfcuagcsa	423	UGCUGAGCGCGCA CACGUAGUAC	558
AD-1685388	csascagUfaGfUfAfcac cgccuuL96	289	asAfsaggCfuGfUfgu acUfaCfugugscsg	424	CGCACACGUAGUA CACCGCCUUG	559
AD-1685406	cscsgggUfaGfGfAfgg ccacaauL96	290	asUfsuguGfgCfCfu ccUfgAfcggscsa	425	UGCCGGGUCAGGA AGGCCACAAA	560
AD-1685418	gsgscacAfaAfGfAfgc gcgugauL96	291	asUfscacGfcCfGfcu cuUfuGfuggcsusu	426	AAGGCCACAAAGA GCGGCGUGAG	561
AD-1685469	cscsacacCfaCfGfAfac cguugcuL96	292	asGfscacCfuGfCfu cgUfgGfugugsasg	427	CUCCACACCACGA AGCCGUUGCC	562
AD-1685491	asgscaggAfaGfGfCfugu gccuguL96	293	asAfscagGfcAfCfag ccUfuCfugcgsusg	428	GCAGCAGGAAGGC UGUGCCUGUG	563
AD-1685503	cscsugugGfcCfCfGfcga agucuuL96	294	asAfsagaCfuUfCfgc ggGfcCfacaggscsa	429	UGCCUGUGGCCCCG CGAAGUCUUC	564

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1685512	csgscgaaGfuCfUfUfcca gcucaguL96	295	asCfsugaGfcUfGfga agAfcUfucgsgsgsg	430	CCCGCGAAGUCUU CCAGCUCAGC	565
AD-1685528	uscsagcaGfuGfUfCfucg uucccuL96	296	asAfsaggAfaCfGfag acAfcUfucgagsgsc	431	GCUCAGCAGUGUC UCGUUCCCUG	566
AD-1685538	gscsagacCfGfCfAfucc uucugguL96	297	asCfscagAfaGfGfau guCfGfucgcsusa	432	UAGCAGACCGACA UCCUUCUGGG	567
AD-1685549	uscscuucUfgGfGfCfua cagccuuL96	298	asAfsggcUfgUfAfgg ccCfaGfaaggasusg	433	CAUCCUUCUGGGC CUACAGCCUG	568
AD-1685561	usascagcCfuGfCfCfucu uuucuguL96	299	asCfsagaAfaAfGfag gcAfgGfcuguasgsg	434	CCUACAGCCUGCC UCUUUUCUGC	569
AD-1685570	cscsucuuUfuCfUfGfccu gggaguL96	300	asAfsucCfcAfGfgc agAfaAfagaggscsa	435	UGCCUCUUUUCUG CCUGGGAGUC	570
AD-1685587	asgsuccuGfaCfUfUfcca cgaggauL96	301	asUfscuCfGfUfGfga agUfcAfggacusesc	436	GGAGUCCUGACUU CCACGAGGAC	571
AD-1685599	csuscaaCfaCfAfAfcuc cuucuuL96	302	asAfsagaAfgGfAfgu ugUfgUfuagsgsgsu	437	ACCUCAAACACAA CUCCUUCUUG	572
AD-1685610	csusccuuCfuUfGfGfaac ccagauL96	303	asAfsucuGfgGfUfuc caAfgAfggagsusu	438	AACUCCUUCUUGG AACCCAGAUC	573
AD-1685614	usgscuccCfaGfUfCfagu ugaccuuL96	304	asAfsnguCfaAfCfug acUfgGfgagcagsgsg	439	CCUGCUCUCCAGUC AGUUGACCUG	574
AD-1685627	cscsuccuUfcCfCfAfgag cucaguL96	305	asAfsucgAfgCfUfcu ggGfaAfggagsgsc	440	GGCCUCCUCCCA GAGCUCAGUG	575
AD-1685637	asgsagcuCfaGfUfGfgac acagaauL96	306	asUfsucuGfuGfUfcc acUfgAfgcucusgsg	441	CCAGAGCUCAGUG GACACAGAAA	576
AD-1685648	ascscuaCfaAfGfGfauc cuuggcuL96	307	asGfscuAfgGfAfu cuUfgUfagggusesc	442	GGACCCUACAAGG AUCCUUGGCA	577
AD-1685664	usgsgcagGfaAfAfGfcag ggauuguL96	308	asCfsaauCfcCfUfgc uuUfcCfugccasag	443	CUUGGCAGGAAAG CAGGGAUUGU	578
AD-1685673	asgscaggGfaUfUfGfug uucuuuuL96	309	asAfsaauGfaAfCfac aaUfcCfcugcususu	444	AAAGCAGGGAUUG UGUUCAUUUG	579
AD-1685689	asusuugaGfgGfUfUfuc acugucauL96	310	asUfsgacAfgUfGfaa acCfcUfcaausgsa	445	UCAUUUGAGGGUU UCACUGUCAG	580
AD-1685699	ususcacuGfuCfAfGfuga gagucuuL96	311	asAfsagcUfcUfCfac ugAfcAfgugaasasc	446	GUUUCACUGUCAG UGAGAGUCUC	581
AD-1685708	asgsugagAfgUfCfUfcag cuuccauL96	312	asUfsggaAfgCfUfga gaCfuCfucacusgsa	447	UCAGUGAGAGUCU CAGCUUCCAU	582
AD-1685717	csuscagcUfuCfCfAfugc aacuguL96	313	asAfsagcUfuGfCfau ggAfaGfcugagsasc	448	GUCUCAGCUUCCA UGCAACUGUC	583
AD-1685726	csasugcaAfcUfGfUfcca ucagguL96	314	asCfscguGfaUfGfga caGfuUfgcaugsgsa	449	UCCAUGCAACUGU CCAUCACGGC	584
AD-1685735	gsusccauCfaCfGfGfcug caacuguL96	315	asCfsaguUfgCfAfgc cgUfgAfggacgsag	450	CUGUCCAUCACGG CUGCAACUGA	585
AD-1685744	gsgscugcAfaCfUfGfaaa ucagaguL96	316	asCfsucuGfaUfUfuc agUfuGfcagccsgsu	451	ACGGCUGCAACUG AAAUCAGAGC	586
AD-1685770	csascagcGfcAfCfCfaga agcuauL96	317	asUfsuagCfuUfCfug guGfcGfcugugsusc	452	GACACAGCGCACC AGAAGCUAAA	587
AD-1685779	cscsagaaGfcUfAfAfagu cuugauL96	318	asAfsucaAfgAfCfu uaGfcUfucggsusg	453	CACCAGAAGCUAA AGUCUUGAUG	588

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1685788	asasagucUfuGfAfUfgcc aucaauL96	319	asUfsuugAfuGfGfca ucAfaGfacuusasg	454	CUAAAGUCUUGAU GCCAUCAAAG	589
AD-1685798	usgsccauCfaAfAfGfgac aucccuL96	320	asAfsgggAfuGfUfcc uuUfgAfuggcasusc	455	GAUGCCAUCAAAG GACAUCCCUG	590
AD-1685810	uscsucugUfcAfCfGfucc acuaauL96	321	asAfsuuaGfuGfGfac guGfaCfagagasusc	456	CAUCUCUGUCACG UCCACUAAUC	591
AD-1685826	usasaucgGfcAfAfAfagg agaaaL96	322	asUfsuuuCfuCfCfu uuGfcCfgaauasgsu	457	ACUAAUCGGCAA AGGAGAAAAG	592
AD-1685838	asasgugaGfaGfAfAfgau gaccuauL96	323	asUfsaggUfcAfUfcu ucUfcUfcacuususu	458	AAAAGUGAGAGA AGAUGACCUA	593
AD-1685847	asasgaugAfcCfUfAfagu gugacuL96	324	asAfsugcAfcAfCfu agGfuCfauuuscusu	459	AGAAGAUGACCUA AGUGUGACUG	594
AD-1685856	usasagugUfgAfCfUfgca gcaggcuL96	325	asGfscuGfcUfGfca guCfaCfauuasgsg	460	CCUAAGUGUGACU GCAGCAGGCA	595
AD-1685869	asgscaggCfaGfCfUfcug gaaaauL96	326	asAfsuuuUfcCfAfga gcUfgCfcugcusgsc	461	GCAGCAGGCAGCU CUGGAAAAUG	596
AD-1685878	csuscuggAfaAfAfUfgaa gccagauL96	327	asUfscugGfcUfUfca uuUfuCfcagagscsu	462	AGCUCUGGAAAAU GAAGCCAGAG	597
AD-1685889	gsasagccAfgAfGfCfagu gagccauL96	328	asUfsggcUfcAfCfug cuCfuGfgcuucsasu	463	AUGAAGCCAGAGC AGUGAGCCAG	598
AD-1685898	cscsgaccAfaGfGfAfgga aggaaaL96	329	asUfsuucCfuUfCfcu ccUfuGfgucggsasg	464	CUCCGACCAAGGA GGAAGGAAAG	599
AD-1685902	gsasggaaGfgAfAfAfga gcagauL96	330	asGfsaucUfgCfUfcu uuCfcUfuccucscsu	465	AGGAGGAAGGAA AGAGCAGAUCC	600
AD-1685912	asgsagcaGfaUfCfCfcag guuuguL96	331	asAfscaaAfcCfUfgg gaUfcUfgcucususu	466	AAAGAGCAGAUCC CAGGUUUGUA	601
AD-1685921	cscscaggUfuUfGfUfaac agaaaL96	332	asUfsuuuCfuGfUfua caAfaCfcugggsasu	467	AUCCCAGGUUUGU AACAGAAAAC	602
AD-1685931	usasacagAfaAfAfCfacc acuaauL96	333	asUfsuuaGfuGfGfug uuUfuCfuguuascsa	468	UGUAACAGAAAAC ACCACUAAAG	603
AD-1685935	csasgcacAfgGfAfGfaga accaccuL96	334	asGfsgugGfuUfCfuc ucCfuGfugcugsgsg	469	CCCAGCACAGGAG AGAACCACCC	604
AD-1685956	asgscccaGfaAfGfUfucc agggaauL96	335	asUfsuccCfuGfGfaa cuUfcUfgggcusgsg	470	CCAGCCCAGAAGU UCCAGGGAAG	605
AD-1685969	csasgggaAfgGfAfAfcuc uccgguL96	336	asAfsccgGfaGfAfgu ucCfuUfcccugsgsa	471	UCCAGGGAAGGAA CUCUCCGGUC	606
AD-1685983	uscscgguCfcAfCfCfaug gaguacuL96	337	asGfsuacUfcCfAfg guGfgAfcggasgsa	472	UCUCCGGUCCACC AUGGAGUACC	607
AD-1685994	asusggagUfaCfCfUfcuc agcucuL96	338	asAfsagCfuGfAfga ggUfaCfuccausgsg	473	CCAUGGAGUACCU CUCAGCUCUG	608
AD-1686007	ususacucAfgGfUfCfagu aucuaauL96	339	asUfsuagAfuAfCfug acCfuGfaguaasgsu	474	ACUUACUCAGGUC AGUAUCUAAU	609
AD-1686016	uscsaguaUfcUfAfAfua aagcucuL96	340	asGfsagCfuAfUfau uaGfaUfacugasasc	475	GGUCAGUAUCUAA UAUAAGCUCG	610
AD-1686028	asusaagcUfcGfGfAfguu uggacguL96	341	asCfsgucCfaAfAfcu ccGfaGfcuausasu	476	AUAUAAGCUCGGA GUUUGGACGG	611
AD-1686053	uscsggaCfcUfCfAfgcu ccaccuL96	342	asUfsgguGfgAfGfcu gaGfgUfcagascsc	477	GGUCUGGACCUCA GCUCCACCAC	612

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1686057	asgscgacCfuUfUfCfcgu gucuguL96	343	asAfsagAfcAfCfagg aaAfgGfucgusgsg	478	CCAGCGACCUUUC CGUGUCUGUG	613
AD-1686066	uscscgugUfcUfGfUfga ucacaaguL96	344	asCfsuugUfgAfUfca caGfaCfacggasasa	479	UUUCCGUGUCUGU GAUCACAAGC	614
AD-1686075	gsusgaucAfcAfAfGfcg gaccauL96	345	asGfsaugGfuCfCfge uuGfuGfaucacsag	480	CUGUGAUCACAAG CGGACCAUCC	615
AD-1686086	csgsgaccAfuCfCfGfgaa aggccuL96	346	asAfsaggCfuUfUfcc ggAfuGfguccgscsu	481	AGCGGACCAUCCG GAAAGGCCUG	616
AD-1686098	asasaggCfuGfAfCfagc ugccacuL96	347	asGfsuggCfaGfCfug ucAfgGfccuuuscsc	482	GGAAAGGCCUGAC AGCUGCCACC	617
AD-1686116	cscsaggaGfcUfGfCfuag ccaaaguL96	348	asCfsuuuGfgCfUfag caGfcUfccuggscsg	483	CGCCAGGAGCUGC UAGCCAAAGC	618
AD-1686126	csusagccAfaAfGfCfauu ggagacuL96	349	asGfsucuCfcAfAfug cuUfuGfgcuagscsa	484	UGCUAGCCAAAGC AUUGGAGACC	619
AD-1686136	csasuuggAfgAfCfCfua cugcuguL96	350	asCfsagcAfgUfAfgg guCfuCfcaugscsu	485	AGCAUUGGAGACC CUACUGCUGA	620
AD-1686147	csusacugCfuGfAfAfug gagugcuL96	351	asAfsagcaCfuCfCfau ucAfgCfaguagsgsg	486	CCCUACUGCUGAA UGGAGUGCUA	621
AD-1686159	gsagsagCfuAfAfCfccu ggugcuL96	352	asAfsagcaCfcAfGfgg uuAfgCfaccucsas	487	AUGGAGUGCUAAC CCUGGUGCUA	622
AD-1686171	csusggugCfuAfGfAfgg aggauL96	353	asCfscauCfcUfCfcuc uAfgCfaccagsgsg	488	CCUGGUGCUAGA GGAGGAUGGA	623
AD-1686181	asgsgaggAfuGfGfAfac ugcaguguL96	354	asCfsacuGfcAfGfu ccAfuCfcuccscsu	489	AGAGGAGGAUGG AACUGCAGUGG	624
AD-1686197	asgsuggaCfaGfUfGfagg acuucuL96	355	asAfsagaaGfuCfCfuc acUfgUfccacugsc	490	GCAGUGGACAGUG AGGACUUCUU	625
AD-1686207	gsasggacUfuCfUfUfcca gcugcuL96	356	asAfsagcaGfcUfGfga agAfaGfuccucsc	491	GUGAGGACUUCUU CCAGCUGCUG	626
AD-1686216	ususcagCfuGfCfUfgga ggaugauL96	357	asUfscacuCfcUfCfca gcAfgCfuggaasgsa	492	UCUCCAGCUGCU GGAGGAUGAC	627
AD-1686225	csusggagGfaUfGfAfcac gugccuL96	358	asAfsaggCfcGfUfgu caUfcCfuccagscsa	493	UGCUGGAGGAUGA CACGUGCCUG	628
AD-1686242	cscsugauGfgUfGfUfug cagucuguL96	359	asCfsagaCfuGfCfaac aCfcAfucaggscsa	494	UGCCUGAUGGUGU UGCAGUCUGG	629
AD-1686251	gsusugcaGfuCfUfGfgu cagagcuL96	360	asAfsagcuCfuGfAfcc agAfcUfgcaacsasc	495	GUGUUGCAGUCUG GUCAGAGCUG	630
AD-1686265	asgsagcuGfgAfGfCfccu acaagguL96	361	asCfscuuGfuAfGfgg cuCfcAfgcucsgsa	496	UCAGAGCUGGAGC CCUACAAGGA	631
AD-1686274	gscscuacCfaAfGfGfagu ggaguguL96	362	asCfsacuCfcAfCfucc uUfgUfaggcsusc	497	GAGCCUACAAGG AGUGGAGUGC	632
AD-1686286	gsusggagUfgCfUfGfuc auauggcuL96	363	asGfsccaUfaUfGfac agCfaCfuccacsusc	498	GAGUGGAGUGCUG UCAUAUGGCC	633
AD-1686319	csasagcaCfaGfCfAfgg acaucguL96	364	asCfsgauGfuCfCfu gcUfgUfgcuugsgsg	499	CCCAAGCACAGCA AGGACAUCGC	634
AD-1686334	csasucgcCfcGfAfUfca ccuuguL96	365	asCfsaaaGfgUfGfaa ucGfgGfcgaugsusc	500	GACAUCGCCCGAU UCACCUUGA	635
AD-1686343	asusucacCfuUfUfGfacg uguacauL96	366	asUfsguaCfaCfGfuc aaAfgGfugauscs	501	CGAUUCACCUUUG ACGUGUACAA	636

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1686354	ascsguguAfcAfAfGfcaa aaccuuL96	367	asAfsgggUfuUfUfgc uuGfuAfcacguscsa	502	UGACGUGUACAAG CAAAACCCUC	637
AD-1686366	asasaaccCfuCfGfAfgac cucuuuuL96	368	asAfsaagAfgGfUfcu cgAfgGfguuuusgsc	503	GCAAAACCCUCGA GACCUCUUUG	638
AD-1686377	gsasccucUfuUfGfGfcag ccugaauL96	369	asUfsucaGfgCfUfgc caAfaGfaggucsusc	504	GAGACCUCUUUGG CAGCCUGAAU	639
AD-1686394	gsasauguCfaAfAfGfcca cauucuuL96	370	asAfsгааUfgUfGfgc uuUfgAfcuuucsasg	505	CUGAAUGUCAAAAG CCACAUUCUA	640
AD-1686403	asgscacAfuUfCfUfacg ggcucuL96	371	asAfsagCfcCfGfua gaAfuGfuggcususu	506	AAAGCCACAUUCU ACGGGCUCUA	641
AD-1686417	gsgscucuAfcUfCfUfaug aguuguuL96	372	asAfscaaCfuCfAfu gaGfuAfgagccscsg	507	CGGGCUCUACUCU AUGAGUUGUG	642
AD-1686426	csusaugaGfuUfGfUfgac uuucaauL96	373	asUfsugaAfaGfUfca caAfcUfcuagsasg	508	CUCUAUGAGUUGU GACUUUCAAG	643
AD-1686435	gsusgacuUfuCfAfAfgg acuuggcuL96	374	asGfsccaAfgUfCfcu ugAfaAfguacsasa	509	UUGUGACUUUCA GGACUUGGCC	644
AD-1686451	usgsgcccAfaAfGfAfaag uacucauL96	375	asUfsgagUfaCfUfu cuUfuGfggccasasg	510	CUUGGCCCAAAGA AAGUACUCAG	645
AD-1686466	ascsucagGfgAfGfCfucc uucguuuL96	376	asAfsacgAfaGfGfag cuCfcCfugagusasc	511	GUACUCAGGGAGC UCCUUCGUUG	646
AD-1686475	gscsucUfcGfUfUfgga ccuccauL96	377	asUfsggaGfgUfCfca acGfaAfggagcsusc	512	GAGCUCCUUCGUU GGACCUCAC	647
AD-1686491	uscscacaCfuGfCfUfgca aggccuuL96	378	asAfsggcCfuUfGfca gcAfgUfguggasgsg	513	CCUCCACACUGCU GCAAGGCCUG	648
AD-1686508	cscsugggCfcAfUfAfu uugcugguL96	379	asCfscagCfaAfCfaua uGfgCfccaggscsc	514	GGCCUGGGCCAUA UGUUGCUGGG	649
AD-1686518	asusguugCfuGfGfGfaa uuuccucuL96	380	asGfsaggAfaAfUfuc ccAfgCfaacausasu	515	AUAUGUUGCUGGG AAUUUCCUCC	650
AD-1686531	ususuccuCfcAfCfCfcuu cgucauuL96	381	asAfsugaCfgAfAfgg guGfgAfggaasusu	516	AAUUUCCUCCACC CUUCGUCAUG	651
AD-1686540	cscsucUfcGfCfAfUfgca guggaguL96	382	asCfsuccAfcUfGfca ugAfcGfaaggsusg	517	CACCCUUCGUCAU GCAGUGGAGG	652
AD-1686543	csusgagcAfgUfGfGfcag cagaaguL96	383	asCfsuucUfgCfUfgc caCfuGfucagscsc	518	GGCUGAGCAGUGG CAGCAGAAGG	653
AD-1686556	csasgaagGfgCfCfGfcu ccauucuL96	384	asGfsaauGfgAfGfgc ggCfcCfuucugscsu	519	AGCAGAAGGGCCG CCUCCAUICC	654
AD-1686565	esgscucCfaUfUfCfua cuaagguL96	385	asCfscuuAfgUfAfgg aaUfgGfaggcgsgsc	520	GCCGCCUCCAUICC CUACUAAAGG	655
AD-1686566	csasgaauCfaUfUfCfaa ccgaccuL96	386	asGfsgucGfgUfUfgg aaUfgAfuucugsgsg	521	CCCAGAAUCAUUC CAACCGACCC	656
AD-1686576	cscsaaccGfaCfCfCfacu gcaaaguL96	387	asCfsuuuGfcAfGfug ggUfcGfguuggsasa	522	UUCCAACCGACCC ACUGCAAAGA	657
AD-1686585	cscsacugCfaAfAfGfacu augacauL96	388	asUfsgucAfuAfGfuc uuUfgCfaguggsgsu	523	ACCCACUGCAAAG ACUAUGACAG	658
AD-1686597	csusaugaCfaGfCfAfuca aauiucuL96	389	asGfsaaaUfuUfGfau gcUfgUfcuagsusc	524	GACUAUGACAGCA UCAAAUUCUA	659
AD-1686606	csasucaaAfuUfUfCfagg accugcuL96	390	asGfscagGfuCfCfug aaAfuUfugaugscsu	525	AGCAUCAAAUUC AGGACCUGCA	660

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1686616	csasggacCfuGfCfAfgacaguacauL96	391	asUfsguaCfuGfUfcu gcAfgGfuccugsasa	526	UUCAGGACCUGCA GACAGUACAG	661
AD-1686626	asgsacagUfaCfAfGfgcu agauaaL96	392	asUfsuauCfuAfGfcc ugUfaCfugucusgsc	527	GCAGACAGUACAG GCUAGAUAAAC	662
AD-1686639	usasgauaAfcCfCfAfcce aauuucL96	393	asGfsaaaUfuGfGfgu ggGfuUfaucuasgsc	528	GCUAGAUAAACCCA CCCAAUUUCC	663
AD-1686649	asgsaacUfuUfCfAfgca uaacgcL96	394	asGfscguUfaUfGfcu gaAfaGfguucusgsu	529	ACAGAACCUUUA GCAUAACGCC	664
AD-1686658	csasgcuaAfaCfGfCfcuc acauccL96	395	asGfsgauGfuGfAfgg cgUfuAfugcugsasa	530	UUCAGCAUAACGC CUCACAUCCC	665
AD-1686669	csuscacaUfcCfCfAfagu cuauacuL96	396	asGfsuauAfgAfCfu ggGfaUfgugagsgsc	531	GCCUCACAUCCCA AGUCUAUACC	666
AD-1686680	asgsucuaUfaCfCfCfuua ccugaauL96	397	asUfsucaGfgUfAfag ggUfaUfagacusug	532	CAAGUCUAUACCC UUACCUGAAG	667
AD-1686693	ascscugaAfgAfAfUfgcu guucuuL96	398	asAfsagaAfcAfGfca uuCfuUfcaggusasa	533	UUACCUGAAGAAU GCUGUUCUUU	668
AD-1686709	uscsuuucCfuAfGfCfcac cuuucuuL96	399	asAfsгааAfgGfUfgg cuAfgGfaagasasc	534	GUUCUUUCCUAGC CACCUUUCUG	669
AD-1686720	csasccuuUfcUfGfGfccu cccacuL96	400	asAfsugGfgAfGfgc caGfaAfgaggugsgsc	535	GCCACCUUUCUGG CCUCCCACUU	670
AD-1686729	gsgscucCfcAfCfUfugc ccugaauL96	401	asUfsucaGfgGfCfaa guGfgGfaggccsasg	536	CUGGCCUCCCACU UGCCCUGAAA	671
AD-1686738	csusugccCfuGfAfAfagg ccaagauL96	402	asUfscuuGfgCfCfu ucAfgGfgcaagsug	537	CACUUGCCCUGAA AGGCCAAGAU	672
AD-1686747	asasaggcCfaAfGfAfuca agaauL96	403	asAfscauCfuUfGfau cuUfgGfccuuuscsa	538	UGAAAGGCCAAGA UCAAGAUGUC	673
AD-1686751	asgsgeauCfuUfGfAfucc cagccuuL96	404	asAfsggcUfgGfGfau caAfgAfugccusgsg	539	CCAGGCAUCUUGA UCCCAGCCUG	674
AD-1686761	asuscccaGfcCfUfGfacu gcugcuL96	405	asAfsgeaGfcAfGfuc agGfcUfgggauscsa	540	UGAUCCCAGCCUG ACUGCUGCUA	675
AD-1686770	usgsacugCfuGfCfUfaca ucuaauL96	406	asAfsuuaGfaUfGfua gcAfgCfagucasgsg	541	CCUGACUGCUGCU ACAUCUAAUC	676
AD-1686781	gscscuccUfgUfCfCfua aacuccL96	407	asGfsgagUfuUfAfgg gaCfaGfgaggcsasu	542	AUGCCUCCUGUCC CUAACUCCC	677
AD-1686783	asgscauaCfuGfAfUfgac agcccuL96	408	asAfsgggCfuGfUfca ucAfgUfaugcusgsg	543	CCAGCAUACUGAU GACAGCCCUC	678
AD-1686794	gsascagcCfcUfCfUfcug acuuauL96	409	asUfsaaaGfuCfAfga gaGfgGfcugucsasu	544	AUGACAGCCCUCU CUGACUUUAC	679
AD-1686803	csuscugaCfuUfUfAfcu ugagauL96	410	asAfsucuCfaAfGfgu aaAfgUfcagagsasg	545	CUCUCUGACUUUA CCUUGAGAUC	680
AD-1686813	ascscuugAfgAfUfCfug ucucauuL96	411	asAfsugaAfgAfCfag auCfuCfaaggusasa	546	UUACCUUGAGAUC UGUCUUCUAU	681

TABLE 5. Unmodified Sense and Antisense Strand Sequences of Human CIDEA dsRNA Agents

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1699864.1	GUCCUGGGCUG AGAUGCCAGU	682	1269-1289	ACUGGGAUCUCAG CCCAGGACAA	1130	1267-1289
AD-1699867.1	CUGGGCUGAGA UCCCAGGUUU	683	1272-1292	AAACCUGGGAUCU CAGCCCAGGA	1131	1270-1292
AD-1699868.1	UGGGCUGAGAU CCCAGGUUUU	684	1273-1293	AAAACCTGGGATC UCAGCCCAGG	1132	1271-1293
AD-1699869.1	GGGCUGAGAUC CCAGGUUUGU	685	1274-1294	ACAAACCUGGGAU CUCAGCCCAG	1133	1272-1294
AD-1699870.1	GGCUGAGAUGC CAGGUUUGUU	686	1275-1295	AACAAACCUGGGA UCUCAGCCCA	1134	1273-1295
AD-1699871.1	GCUGAGAUGCC AGGUUUGUAU	687	1276-1296	ATACAAACCUGGG AUCUCAGCCC	1135	1274-1296
AD-1699872.1	CUGAGAUGCCA GGUUUGUAAU	688	1277-1297	ATUACAAACCUGG GAUCUCAGCC	1136	1275-1297
AD-1699873.1	UGAGAUGCCAG GUUUGUAACU	689	1278-1298	AGUUACAAACCTG GGAUCUCAGC	1137	1276-1298
AD-1699875.1	AGAUGCCAGGU UUGUAACAGU	690	1280-1300	ACUGTUACAAACC UGGGAUCUCA	1138	1278-1300
AD-1699882.1	AGGUUUGUAAC AGAAAACACU	691	1287-1307	AGUGTUTUCUGTU ACAAACCUGG	1139	1285-1307
AD-1699891.1	ACAGAAAACAC CACUAAAGCU	692	1296-1316	AGCUTUAGUGGTG UUUUCUGUUA	1140	1294-1316
AD-1699892.1	CAGAAAACACC ACUAAAGCCU	693	1297-1317	AGGCTUTAGUGGU GUUUUCUGUU	1141	1295-1317
AD-1699904.1	AGAACCACCCAG CCCAGAAGU	694	1329-1349	ACUUCUGGGCUGG GUGGUUCUCU	1142	1327-1349
AD-1699907.1	ACCACCCAGCCC AGAAGUUCU	695	1332-1352	AGAACUTCUGGGC UGGGUGGUUC	1143	1330-1352
AD-1699948.1	CACCAUGGAGU ACCUCUCAGU	696	1373-1393	ACUGAGAGGUAC UCCAUGGUGGA	1144	1371-1393
AD-1699949.1	ACCAUGGAGUA CCUCUCAGCU	697	1374-1394	AGCUGAGAGGUA CUCCAUGGUGG	1145	1372-1394
AD-1699951.1	CAUGGAGUACC UCUCAGCUCU	698	1376-1396	AGAGCUGAGAGG UACUCCAUGGU	1146	1374-1396
AD-1699964.1	CUUACUCAGGU CAGUAUCUAU	699	1409-1429	ATAGAUACUGACC UGAGUAAGUC	1147	1407-1429
AD-1699967.1	ACUCAGGUCAG UAUCUAAUAU	700	1412-1432	ATAUTAGAUACTG ACCUGAGUAA	1148	1410-1432
AD-1699970.1	CAGGUCAGUAU CUAAUAUAAU	701	1415-1435	ATUATATUAGATA CUGACCUGAG	1149	1413-1435
AD-1699971.1	AGGUCAGUAUC UAAUAUAAGU	702	1416-1436	ACUUAUAUUAGA UACUGACCUGA	1150	1414-1436
AD-1699972.1	GGUCAGUAUCU AAUAUAAGCU	703	1417-1437	AGCUTATAUUAGA UACUGACCUG	1151	1415-1437
AD-1699973.1	GUCAGUAUCUA AUUAUAAGCU	704	1418-1438	AAGCTUAUAUUAG AUACUGACCU	1152	1416-1438

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1699976.1	AGUAUCUAAUA UAAGCUCGGU	705	1421-1441	ACCGAGCUUAUUA UAGAUACUGA	1153	1419-1441
AD-1699977.1	GUAUCUAAUAU AAGCUCGGAU	706	1422-1442	ATCCGAGCUUATA UUAGAUACUG	1154	1420-1442
AD-1699978.1	UAUCUAAUAUA AGCUCGGAGU	707	1423-1443	ACUCCGAGCUUAU AUUAGAUACU	1155	1421-1443
AD-1699979.1	AUCUAAUAUAA GCUCGGAGUU	708	1424-1444	AACUCCGAGCUTA UAUUAGAUAC	1156	1422-1444
AD-1699982.1	UAAUAUAAGCU CGGAGUUUGU	709	1427-1447	ACAAACTCCGAGC UUAUAUUAGA	1157	1425-1447
AD-1699983.1	AAUAUAAGCUC GGAGUUUGGU	710	1428-1448	ACCAAACUCCGAG CUUAUAUUAG	1158	1426-1448
AD-1699984.1	AUAUAAGCUCG GAGUUUGGAU	711	1429-1449	ATCCAAACUCCGA GCUUAUAUUA	1159	1427-1449
AD-1699985.1	UAUAAGCUCGG AGUUUGGACU	712	1430-1450	AGUCCAAACUCCG AGCUUAUAUU	1160	1428-1450
AD-1699986.1	AUAAGCUCGGA GUUUGGACGU	713	1431-1451	ACGUCCAAACUCC GAGCUUAUAU	1161	1429-1451
AD-1699987.1	UAAGCUCGGAG UUUGGACGGU	714	1432-1452	ACCGTCCAAACTC CGAGCUUAUA	1162	1430-1452
AD-1699988.1	AAGCUCGGAGU UUGGACGGAU	715	1433-1453	ATCCGUCCAAACU CCGAGCUUAU	1163	1431-1453
AD-1699989.1	AGCUCGGAGUU UGGACGGAGU	716	1434-1454	ACUCCGTCCAAAC UCCGAGCUUA	1164	1432-1454
AD-1699990.1	GCUCGGAGUUU GGACGGAGGU	717	1435-1455	ACCUCGUCCAAAC CUCCGAGCUU	1165	1433-1455
AD-1700015.1	AGCGACCUUUC GUGUCUGUU	718	1480-1500	AACAGACACGGAA AGGUCGCUUG	1166	1478-1500
AD-1700035.1	GAUCACAAGCG GACCAUCCGU	719	1500-1520	ACGGAUGGUCCGC UUGUGAUCAC	1167	1498-1520
AD-1700037.1	UCACAAGCGGA CCAUCCGGAU	720	1502-1522	ATCCGGAUGGUCC GCUUGUGAUC	1168	1500-1522
AD-1700038.1	CACAAGCGGACC AUCCGGAAU	721	1503-1523	ATUCCGGAUGGTC CGCUUGUGAU	1169	1501-1523
AD-1700039.1	ACAAGCGGACC AUCCGGAAAU	722	1504-1524	ATUCCGGAUGGU CCGCUUGUGA	1170	1502-1524
AD-1700040.1	CAAGCGGACCA UCCGGAAAGU	723	1505-1525	ACUUTCCGGAUGG UCCGCUUGUG	1171	1503-1525
AD-1700041.1	AAGCGGACCAU CCGGAAAGGU	724	1506-1526	ACCUTUCCGGATG GUCCGCUUGU	1172	1504-1526
AD-1700077.1	GGAGCUGCUAG CCAAAGCAUU	725	1547-1567	AAUGCUTUGGCTA GCAGCUCCUG	1173	1545-1567
AD-1700080.1	GCUGCUAGCCA AAGCAUUGGU	726	1550-1570	ACCAAUGC UUUGG CUAGCAGCUC	1174	1548-1570
AD-1700081.1	CUGCUAGCCAA AGCAUUGGAU	727	1551-1571	ATCCAATGCUUTG GCUAGCAGCU	1175	1549-1571
AD-1700082.1	UGCUAGCCAAA GCAUUGGAGU	728	1552-1572	ACUCCAAUGCUTU GGCUAGCAGC	1176	1550-1572

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700083.1	GCUAGCCAAAG CAUUGGAGAU	729	1553-1573	ATCUCCAAUGCTU UGGCUAGCAG	1177	1551-1573
AD-1700091.1	AAGCAUUGGAG ACCCUACUGU	730	1561-1581	ACAGTAGGGUUTC CAAUGCUUUG	1178	1559-1581
AD-1700092.1	AGCAUUGGAGA CCCUACUGCU	731	1562-1582	AGCAGUAGGGUC UCCAAUGCUUU	1179	1560-1582
AD-1700094.1	CAUUGGAGACC CUACUGCUGU	732	1564-1584	ACAGCAGUAGGG UCUCCAAUGCU	1180	1562-1584
AD-1700097.1	UGGAGACCCUA CUGCUGAAUU	733	1567-1587	AAUUCAGCAGUA GGGUCUCCAAU	1181	1565-1587
AD-1700099.1	GAGACCCUACU GCUGAAUGGU	734	1569-1589	ACCATUCAGCAGU AGGGUCUCCA	1182	1567-1589
AD-1700100.1	AGACCCUACUGC UGAAUGGAU	735	1570-1590	ATCCAUTCAGCAG UAGGGUCUCC	1183	1568-1590
AD-1700101.1	GACCCUACUGCU GAAUGGAGU	736	1571-1591	ACUCCATUCAGCA GUAGGGUCUC	1184	1569-1591
AD-1700104.1	CCUACUGCUGA AUGGAGUGCU	737	1574-1594	AGCACUCCAUUCA GCAGUAGGGU	1185	1572-1594
AD-1700109.1	UGCUGAAUGGA GUGCUAACCU	738	1579-1599	AGGUTAGCACUCC AUUCAGCAGU	1186	1577-1599
AD-1700112.1	UGAAUGGAGUG CUAACCCUGU	739	1582-1602	ACAGGGTUAGCAC UCCAUUCAGC	1187	1580-1602
AD-1700113.1	GAAUGGAGUGC UAACCCUGGU	740	1583-1603	ACCAGGGUUAGCA CUCCAUUCAG	1188	1581-1603
AD-1700114.1	AAUGGAGUGCU AACCCUGGUU	741	1584-1604	AACCAGGGUUAGC ACUCCAUUCA	1189	1582-1604
AD-1700115.1	AUGGAGUGCUA ACCCUGGUGU	742	1585-1605	ACACCAGGGUUAG CACUCCAUUC	1190	1583-1605
AD-1700116.1	UGGAGUGCUAA CCCUGGUGCU	743	1586-1606	AGCACCAGGGUTA GCACUCCAUAU	1191	1584-1606
AD-1700121.1	UGCUAACCCUG GUGCUAGAGU	744	1591-1611	ACUCTAGCACCAG GGUUAGCACU	1192	1589-1611
AD-1700122.1	GCUAACCCUGG UGCUAGAGGU	745	1592-1612	ACCUCUAGCACCA GGGUUAGCAC	1193	1590-1612
AD-1700125.1	AACCCUGGUGC UAGAGGAGGU	746	1595-1615	ACCUCCTCUAGCA CCAGGGUUAG	1194	1593-1615
AD-1700148.1	GAACUGCAGUG GACAGUGAGU	747	1618-1638	ACUCACTGUCCAC UGCAGUUGCA	1195	1616-1638
AD-1700149.1	AACUGCAGUGG ACAGUGAGGU	748	1619-1639	ACCUCACUGUCCA CUGCAGUCC	1196	1617-1639
AD-1700156.1	GUGGACAGUGA GGACUUCUUU	749	1626-1646	AAAGAAGUCCUCA CUGUCCACUG	1197	1624-1646
AD-1700158.1	GGACAGUGAGG ACUUCUCCU	750	1628-1648	AGGAAGAAGUCC UCACUGUCCAC	1198	1626-1648
AD-1700159.1	GACAGUGAGGA CUUCUCCAU	751	1629-1649	ATGGAAGAAGUCC UCACUGUCCA	1199	1627-1649
AD-1700160.1	ACAGUGAGGAC UUCUCCAGU	752	1630-1650	ACUGGAAGAAGTC CUCACUGUCC	1200	1628-1650

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700166.1	AGGACUUCUUC CAGCUGCUGU	753	1636-1656	ACAGCAGCUGGAA GAAGUCCUCA	1201	1634-1656
AD-1700167.1	GGACUUCUUC AGCUGCUGGU	754	1637-1657	ACCAGCAGCUGGA AGAAGUCCUC	1202	1635-1657
AD-1700180.1	CUGCUGGAGGA UGACACGUGU	755	1650-1670	ACACGUGUCAUCC UCCAGCAGCU	1203	1648-1670
AD-1700187.1	AGGAUGACACG UGCCUGAUGU	756	1657-1677	ACAUCAGGCACGU GUCAUCCUCC	1204	1655-1677
AD-1700188.1	GGAUGACACGU GCCUGAUGGU	757	1658-1678	ACCATCAGGCACG UGUCAUCCUC	1205	1656-1678
AD-1700189.1	GAUGACACGUG CCUGAUGGUU	758	1659-1679	AACCAUCAGGCAC GUGUCAUCCU	1206	1657-1679
AD-1700190.1	AUGACACGUGC CUGAUGGUGU	759	1660-1680	ACACCATCAGGCA CGUGUCAUCC	1207	1658-1680
AD-1700191.1	UGACACGUGCC UGAUGGUGUU	760	1661-1681	AACACCAUCAGGC ACGUGUCAUC	1208	1659-1681
AD-1700193.1	ACACGUGCCUG AUGGUGUUGU	761	1663-1683	ACAACACCAUCAG GCACGUGUCA	1209	1661-1683
AD-1700213.1	CAGUCUGGUCA GAGCUGGAGU	762	1683-1703	ACUCCAGCUCUGA CCAGACUGCA	1210	1681-1703
AD-1700242.1	GAGUGGAGUGC UGUCAUAUGU	763	1712-1732	ACAUAUGACAGCA CUCCACUCCU	1211	1710-1732
AD-1700243.1	AGUGGAGUGCU GUCAUAUGGU	764	1713-1733	ACCATATGACAGC ACUCCACUCC	1212	1711-1733
AD-1700244.1	GUGGAGUGCUG UCAUAUGGCU	765	1714-1734	AGCCAUAUGACAG CACUCCACUC	1213	1712-1734
AD-1700245.1	UGGAGUGCUGU CAUAUGGCCU	766	1715-1735	AGGCCATAUGACA GCACUCCACU	1214	1713-1735
AD-1700248.1	AGUGCUGUCAU AUGGCCUGGU	767	1718-1738	ACCAGGCCAUATG ACAGCACUCC	1215	1716-1738
AD-1700249.1	GUGCUGUCAUA UGGCCUGGGU	768	1719-1739	ACCCAGGCCAUAU GACAGCACUC	1216	1717-1739
AD-1700250.1	UGCUGUCAUAU GGCCUGGGAU	769	1720-1740	ATCCCAGGCCATA UGACAGCACU	1217	1718-1740
AD-1700252.1	CUGUCAUAUGG CCUGGGACGU	770	1722-1742	ACGUCCCAGGCCA UAUGACAGCA	1218	1720-1742
AD-1700272.1	AGGCCCAAGCAC AGCAAGGAU	771	1746-1766	ATCCTUGCUGUGC UUGGGCCUCU	1219	1744-1766
AD-1700279.1	AGCACAGCAAG GACAUCGCCU	772	1753-1773	AGGCGATGUCCTU GCUGUGCUUG	1220	1751-1773
AD-1700280.1	GCACAGCAAGG ACAUCGCCCCU	773	1754-1774	AGGGCGAUGUCCU UGCUGUGCUU	1221	1752-1774
AD-1700303.1	UCACCUUUGAC GUGUACAAGU	774	1777-1797	ACUUGUACACGTC AAAGGUGAAU	1222	1775-1797
AD-1700309.1	UUGACGUGUAC AAGCAAAACU	775	1783-1803	AGUUTUGCUUGTA CACGUCAAAG	1223	1781-1803
AD-1700312.1	ACGUGUACAAG CAAAACCCUU	776	1786-1806	AAGGGUTUUGCTU GUACACGUCA	1224	1784-1806

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700314.1	GUGUACAAGCA AAACCCUCGU	777	1788-1808	ACGAGGGUUUUG CUUGUACACGU	1225	1786-1808
AD-1700315.1	UGUACAAGCAA AACCCUCGAU	778	1789-1809	ATCGAGGGUUUTG CUUGUACACG	1226	1787-1809
AD-1700316.1	GUACAAGCAAA ACCCUCGAGU	779	1790-1810	ACUCGAGGGUUTU GCUUGUACAC	1227	1788-1810
AD-1700317.1	UACAAGCAAAA CCCUCGAGAU	780	1791-1811	ATCUCGAGGGUTU UGCUUGUACA	1228	1789-1811
AD-1700318.1	ACAAGCAAAAC CCUCGAGACU	781	1792-1812	AGUCTCGAGGGTU UUGCUUGUAC	1229	1790-1812
AD-1700323.1	CAAAACCCUCGA GACCUCUUU	782	1797-1817	AAAGAGGUCUCG AGGGUUUUGCU	1230	1795-1817
AD-1700324.1	AAAACCCUCGA GACCUCUUU	783	1798-1818	AAAAGAGGUCUC GAGGGUUUUGC	1231	1796-1818
AD-1700325.1	AAACCCUCGAG ACCUCUUUGU	784	1799-1819	ACAAAGAGGUCTC GAGGGUUUUG	1232	1797-1819
AD-1700327.1	ACCCUCGAGACC UCUUUGGCU	785	1801-1821	AGCCAAAGAGGTC UCGAGGGUUU	1233	1799-1821
AD-1700328.1	CCCUCGAGACCU CUUUGGCAU	786	1802-1822	ATGCCAAAGAGGU CUCGAGGGUU	1234	1800-1822
AD-1700329.1	CCUCGAGACCUC UUUGGCAGU	787	1803-1823	ACUGCCAAAGAGG UCUCGAGGGU	1235	1801-1823
AD-1700336.1	ACCUCUUUGGC AGCCUGAAUU	788	1810-1830	AAUUCAGGCUGCC AAAGAGGUCU	1236	1808-1830
AD-1700339.1	UCUUUGGCAGC CUGAAUGUCU	789	1813-1833	AGACAUTCAGGCU GCCAAAGAGG	1237	1811-1833
AD-1700344.1	GGCAGCCUGAA UGUCAAGGCU	790	1818-1838	AGCUTUGACAUTC AGGCUGCCAA	1238	1816-1838
AD-1700350.1	CUGAAUGUCAA AGCCACAUUU	791	1824-1844	AAAUGUGGCUUTG ACAUUCAGGC	1239	1822-1844
AD-1700352.1	GAAUGUCAAAAG CCACAUUCUU	792	1826-1846	AAGAAUGUGGCTU UGACAUUCAG	1240	1824-1846
AD-1700353.1	AAUGUCAAAAGC CACAUUCUAU	793	1827-1847	ATAGAATGUGGCU UUGACAUUCA	1241	1825-1847
AD-1700354.1	AUGUCAAAAGCC ACAUUCUACU	794	1828-1848	AGUAGAAUGUGG CUUUGACAUUC	1242	1826-1848
AD-1700355.1	UGUCAAAAGCCA CAUUCUACGU	795	1829-1849	ACGUAGAAUGUG GCUUUGACAUU	1243	1827-1849
AD-1700356.1	GUCAAAAGCCAC AUUCUACGGU	796	1830-1850	ACCGTAGAAUGTG GCUUUGACAU	1244	1828-1850
AD-1700357.1	UCAAAAGCCACA UUCUACGGGU	797	1831-1851	ACCCGUAGAAUGU GGCUUUGACA	1245	1829-1851
AD-1700358.1	CAAAGCCACAU UCUACGGGCU	798	1832-1852	AGCCCGTAGAATG UGGCUUUGAC	1246	1830-1852
AD-1700359.1	AAAGCCACAUU CUACGGGCUU	799	1833-1853	AAGCCCGUAGAAU GUGGCUUUGA	1247	1831-1853
AD-1700365.1	ACAUUCUACGG GCUCUACUCU	800	1839-1859	AGAGTAGAGCCCG UAGAAUGUGG	1248	1837-1859

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700366.1	CAUUCUACGGG CUCUACUCUU	801	1840-1860	AAGAGUAGAGCCC GUAGAAUGUG	1249	1838-1860
AD-1700368.1	UUCUACGGGCU CUACUCUAUU	802	1842-1862	AAUAGAGUAGAG CCCGUAGAAUG	1250	1840-1862
AD-1700369.1	UCUACGGGCUC UACUCUAUGU	803	1843-1863	ACAUAGAGUAGA GCCCGUAGAAU	1251	1841-1863
AD-1700370.1	CUACGGGCUCU ACUCUAUGAU	804	1844-1864	ATCATAGAGUAGA GCCCGUAGAA	1252	1842-1864
AD-1700371.1	UACGGGCUCUA CUCUAUGAGU	805	1845-1865	ACUCAUAGAGUA GAGCCCGUAGA	1253	1843-1865
AD-1700372.1	ACGGGCUCUAC UCUAUGAGUU	806	1846-1866	AACUCATAGAGTA GAGCCCGUAG	1254	1844-1866
AD-1700374.1	GGGCUCUACUC UAUGAGUUGU	807	1848-1868	ACAACUCAUAGAG UAGAGCCCGU	1255	1846-1868
AD-1700375.1	GGCUCUACUCU AUGAGUUGUU	808	1849-1869	AACAACUCAUAGA GUAGAGCCCG	1256	1847-1869
AD-1700376.1	GCUCUACUCUA UGAGUUGUGU	809	1850-1870	ACACAACUCAUAG AGUAGAGCCC	1257	1848-1870
AD-1700378.1	UCUACUCUAUG AGUUGUGACU	810	1852-1872	AGUCACAACUCAU AGAGUAGAGC	1258	1850-1872
AD-1700383.1	UCUAUGAGUUG UGACUUUCAU	811	1857-1877	ATGAAAGUCACAA CUCAUAGAGU	1259	1855-1877
AD-1700384.1	CUAUGAGUUGU GACUUUCAAU	812	1858-1878	ATUGAAAGUCACA ACUCAUAGAG	1260	1856-1878
AD-1700385.1	UAUGAGUUGUG ACUUUCAAGU	813	1859-1879	ACUUGAAAGUCAC AACUCAUAGA	1261	1857-1879
AD-1700386.1	AUGAGUUGUGA CUUUCAAGGU	814	1860-1880	ACCUTGAAAGUCA CAACUCAUAG	1262	1858-1880
AD-1700387.1	UGAGUUGUGAC UUUCAAGGAU	815	1861-1881	ATCCTUGAAAGTC ACAACUCAUA	1263	1859-1881
AD-1700388.1	GAGUUGUGACU UUCAAGGACU	816	1862-1882	AGUCCUTGAAAGU CACAACUCAU	1264	1860-1882
AD-1700408.1	UUGGCCCAAAG AAAGUACUCU	817	1882-1902	AGAGTACUUUCTU UGGGCCAAAGU	1265	1880-1902
AD-1700410.1	GGCCCAAAGAA AGUACUCAGU	818	1884-1904	ACUGAGTACUUTC UUUGGGCCAA	1266	1882-1904
AD-1700411.1	GCCCAAAGAAA GUACUCAGGU	819	1885-1905	ACCUGAGUACUTU CUUUGGGCCA	1267	1883-1905
AD-1700413.1	CCAAAGAAAGU ACUCAGGGAU	820	1887-1907	ATCCCUGAGUACU UUCUUUGGGC	1268	1885-1907
AD-1700414.1	CAAAGAAAGUA CUCAGGGAGU	821	1888-1908	ACUCCCTGAGUAC UUUCUUUGGG	1269	1886-1908
AD-1700422.1	GUACUCAGGGA GCUCCUUCGU	822	1896-1916	ACGAAGGAGCUCC CUGAGUACUU	1270	1894-1916
AD-1700423.1	UACUCAGGGAG CUCCUUCGUU	823	1897-1917	AACGAAGGAGCTC CCUGAGUACU	1271	1895-1917
AD-1700424.1	ACUCAGGGAGC UCCUUCGUUU	824	1898-1918	AAACGAAGGAGC UCCUGAGUAC	1272	1896-1918

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700425.1	CUCAGGGAGCU CCUUCGUUGU	825	1899-1919	ACAACGAAGGAGC UCCCUGAGUA	1273	1897-1919
AD-1700426.1	UCAGGGAGCUC CUUCGUUGGU	826	1900-1920	ACCAACGAAGGAG CUCCCUGAGU	1274	1898-1920
AD-1700427.1	CAGGGAGCUCC UUCGUUGGAU	827	1901-1921	ATCCAACGAAGGA GCUCCCUGAG	1275	1899-1921
AD-1700428.1	AGGGAGCUCCU UCGUUGGACU	828	1902-1922	AGUCCAACGAAGG AGCUCCCUGA	1276	1900-1922
AD-1700439.1	UCGUUGGACCU CCACACUGCU	829	1913-1933	AGCAGUGUGGAG GUCCAACGAAG	1277	1911-1933
AD-1700461.1	CAAGGCCUGGG CCAUAUGUUU	830	1935-1955	AAACAUAUGGCCC AGGCCUUGCA	1278	1933-1955
AD-1700462.1	AAGGCCUGGGC CAUAUGUUGU	831	1936-1956	ACAACATAUGGCC CAGGCCUUGC	1279	1934-1956
AD-1700464.1	GGCCUGGGCCA UAUGUUGCUU	832	1938-1958	AAGCAACAUAUG GCCCAGGCCUU	1280	1936-1958
AD-1700465.1	GCCUGGGCCAU AUGUUGCUGU	833	1939-1959	ACAGCAACAUAATG GCCCAGGCCU	1281	1937-1959
AD-1700468.1	UGGGCCAUAUG UUGCUGGGAU	834	1942-1962	ATCCCAGCAACAU AUGGCCCAGG	1282	1940-1962
AD-1700473.1	CAUAUGUUGCU GGGAAUUUCU	835	1947-1967	AGAAAUTCCCAGC AACAU AUGGC	1283	1945-1967
AD-1700474.1	AUAUGUUGCUG GGAAUUUCCU	836	1948-1968	AGGAAATUCCCAG CAACAUAUGG	1284	1946-1968
AD-1700476.1	AUGUUGCUGGG AAUUUCCUCU	837	1950-1970	AGAGGAAAUUCCC AGCAACAUAU	1285	1948-1970
AD-1700477.1	UGUUGCUGGGA AUUUCCUCCU	838	1951-1971	AGGAGGAAAUUC CCAGCAACAUA	1286	1949-1971
AD-1700479.1	UUGCUGGGAAU UUCCUCCACU	839	1953-1973	AGUGGAGGAAAT UCCCAGCAACA	1287	1951-1973
AD-1700482.1	CUGGGAAUUUC CUCCACCCUU	840	1956-1976	AAGGGUGGAGGA AAUUCCCAGCA	1288	1954-1976
AD-1700485.1	GGAUUUCCUC CACCCUUCGU	841	1959-1979	ACGAAGGGUGGA GGAAAUUCCA	1289	1957-1979
AD-1700486.1	GAAUUUCCUCC ACCCUUCGUU	842	1960-1980	AACGAAGGGUGG AGGAAAUUCCC	1290	1958-1980
AD-1700487.1	AAUUUCCUCCAC CCUUCGUCU	843	1961-1981	AGACGAAGGGUG GAGGAAAUUC	1291	1959-1981
AD-1700488.1	AUUUCCUCCACC CUUCGUCAU	844	1962-1982	ATGACGAAGGGTG GAGGAAAUUC	1292	1960-1982
AD-1700489.1	UUUCCUCCACCC UUCGUCAUU	845	1963-1983	AAUGACGAAGGG UGGAGGAAAUU	1293	1961-1983
AD-1700490.1	UUCCUCCACCCU UCGUCAUGU	846	1964-1984	ACAUGACGAAGG GUGGAGGAAAU	1294	1962-1984
AD-1700517.1	AAGGGCCGCCUC CAUUCUUAU	847	2013-2033	ATAGGAAUGGAG GCGGCCCUUCU	1295	2011-2033
AD-1700519.1	GGGCCGCCUCCA UUCUACUU	848	2015-2035	AAGUAGGAAUGG AGGCGGCCCUU	1296	2013-2035

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700520.1	GGCCGCCUCCAU UCCUACUAU	849	2016-2036	ATAGTAGGAAUGG AGGCGGCCCU	1297	2014-2036
AD-1700522.1	CCGCCUCCAUUC CUACUAAGU	850	2018-2038	ACUUAGTAGGAAU GGAGGCGGCC	1298	2016-2038
AD-1700542.1	CCCACUGCAAAG ACUAUGACU	851	2076-2096	AGUCAUAGUCUTU GCAGUGGGUC	1299	2074-2096
AD-1700544.1	CACUGCAAAGA CUAUGACAGU	852	2078-2098	ACUGTCAUAGUCU UUGCAGUGGG	1300	2076-2098
AD-1700550.1	AAAGACUAUGA CAGCAUCAAU	853	2084-2104	ATUGAUGCUGUCA UAGUCUUUGC	1301	2082-2104
AD-1700553.1	GACUAUGACAG CAUCAAUUU	854	2087-2107	AAAUTUGAUGCTG UCAUAGUCUU	1302	2085-2107
AD-1700554.1	ACUAUGACAGC AUCAAUUUU	855	2088-2108	AAAATUTGAUGCU GUCAUAGUCU	1303	2086-2108
AD-1700555.1	CUAUGACAGCA UCAAAUUUCU	856	2089-2109	AGAAAUTUGAUGC UGUCAUAGUC	1304	2087-2109
AD-1700556.1	UAUGACAGCAU CAAUUUCAU	857	2090-2110	ATGAAATUUGATG CUGUCAUAGU	1305	2088-2110
AD-1700557.1	AUGACAGCAUC AAAUUUCAGU	858	2091-2111	ACUGAAAUUUGA UGCUGUCAUAG	1306	2089-2111
AD-1700558.1	UGACAGCAUCA AAUUUCAGGU	859	2092-2112	ACCUGAAAUUUG AUGCUGUCAUA	1307	2090-2112
AD-1700560.1	ACAGCAUCAAA UUUCAGGACU	860	2094-2114	AGUCCUGAAAUTU GAUGCUGUCA	1308	2092-2114
AD-1700563.1	GCAUCAAUUUU CAGGACCUGU	861	2097-2117	ACAGGUCCUGAAA UUUGAUGCUG	1309	2095-2117
AD-1700566.1	UCAAAUUUCAG GACCUGCAGU	862	2100-2120	ACUGCAGGUCCTG AAAUUUGAUG	1310	2098-2120
AD-1700573.1	UCAGGACCUGC AGACAGUACU	863	2107-2127	AGUACUGUCUGCA GGUCCUGAAA	1311	2105-2127
AD-1700575.1	AGGACCUGCAG ACAGUACAGU	864	2109-2129	ACUGTACUGUCTG CAGGUCCUGA	1312	2107-2129
AD-1700576.1	GGACCUGCAGA CAGUACAGGU	865	2110-2130	ACCUGUACUGUCU GCAGGUCCUG	1313	2108-2130
AD-1700578.1	ACCUGCAGACA GUACAGGCUU	866	2112-2132	AAGCCUGUACUGU CUGCAGGUCC	1314	2110-2132
AD-1700580.1	CUGCAGACAGU ACAGGCUAGU	867	2114-2134	ACUAGCCUGUACU GUCUGCAGGU	1315	2112-2134
AD-1700582.1	GCAGACAGUAC AGGCUAGAUU	868	2116-2136	AAUCTAGCCUGTA CUGUCUGCAG	1316	2114-2136
AD-1700623.1	ACGCCUCACAUC CCAAGUCUU	869	2194-2214	AAGACUTGGGATG UGAGGCGUUA	1317	2192-2214
AD-1700670.1	UUCCUAGCCACC UUUCUGGCU	870	2241-2261	AGCCAGAAAGGTG GCUAGGAAAG	1318	2239-2261
AD-1700671.1	UCCUAGCCACCU UUCUGGCCU	871	2242-2262	AGGCCAGAAAGG UGGCUAGGAAA	1319	2240-2262
AD-1700680.1	CCUUUCUGGCCU CCCACUUGU	872	2251-2271	ACAAGUGGGAGG CCAGAAAGGUG	1320	2249-2271

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700681.1	CUUUCUGGCCUC CCACUUGCU	873	2252-2272	AGCAAGTGGGAGG CCAGAAAGGU	1321	2250-2272
AD-1700684.1	UCUGGCCUCCCA CUUGCCCUU	874	2255-2275	AAGGGCAAGUGG GAGGCCAGAAA	1322	2253-2275
AD-1700710.1	GGCAUCUUGAU CCCAGCCUGU	875	2302-2322	ACAGGCTGGGATC AAGAUGCCUG	1323	2300-2322
AD-1700736.1	AAUGCCUCCUG UCCCUAAACU	876	2348-2368	AGUUTAGGGACAG GAGGCAUUGG	1324	2346-2368
AD-1700737.1	AUGCCUCCUGUC CCUAAACUU	877	2349-2369	AAGUTUAGGGACA GGAGGCAUUG	1325	2347-2369
AD-1700738.1	UGCCUCCUGUCC CUAAACUCU	878	2350-2370	AGAGTUTAGGGAC AGGAGGCAUU	1326	2348-2370
AD-1700780.1	CAAACUAACAA AAACAUUUCU	879	2434-2454	AGAAAUGUUUUT GUUAGUUUGAG	1327	2432-2454
AD-1700781.1	AAACUAACAAA AACAUUCCU	880	2435-2455	AGGAAATGUUUTU GUUAGUUUGA	1328	2433-2455
AD-1700782.1	AACUAACAAAA ACAUUCCAU	881	2436-2456	ATGGAAAUGUUTU UGUUAGUUUG	1329	2434-2456
AD-1700791.1	GUCCUGGGCUG AGAUCCAGU	882	1269-1289	ACUGGGAUCUCAG CCCAGGACAA	1330	1267-1289
AD-1700793.1	UGAGAUCCCAG GUUUGUAACU	883	1278-1298	AGUUACAAACCUG GGAUCUCAGC	1331	1276-1298
AD-1700794.1	GAGAUCCCAGG UUUGUAACAU	884	1279-1299	AUGUTACAAACCU GGGAUCUCAG	1332	1277-1299
AD-1700795.1	GAUCCCAGGUU UGUAAACAGAU	885	1281-1301	AUCUGUTACAAAC CUGGGAUCUC	1333	1279-1301
AD-1700796.1	CCAGGUUUGUA ACAGAAAACU	886	1285-1305	AGUUTUCUGUAC AAACCUGGGA	1334	1283-1305
AD-1700797.1	CAGGUUUGUAA CAGAAAACAU	887	1286-1306	AUGUTUTCUGUUA CAAACCUGGG	1335	1284-1306
AD-1700798.1	AGGUUUGUAAC AGAAAACACU	888	1287-1307	AGUGTUTUCUGUU ACAAACCUGG	1336	1285-1307
AD-1700799.1	GGUUUGUAACA GAAAACACCU	889	1288-1308	AGGUGUTUUCUGU UACAAACCUG	1337	1286-1308
AD-1700800.1	UACAGAAAAC ACCACUAAAU	890	1294-1314	AUUUAGTGGUGU UUUCUGUUA	1338	1292-1314
AD-1700801.1	CAGAAAACACC ACUAAAGCCU	891	1297-1317	AGGCTUTAGUGGU GUUUUCUGUU	1339	1295-1317
AD-1700802.1	CACAGGAGAGA ACCACCCAGU	892	1321-1341	ACUGGGTGGUUCU CUCCUGUGCU	1340	1319-1341
AD-1700804.1	GAACCACCCAGC CCAGAAGUU	893	1330-1350	AACUTCTGGGCUG GGUGGUUCUC	1341	1328-1350
AD-1700805.1	AACCACCCAGCC CAGAAGUUU	894	1331-1351	AAACTUCUGGGCU GGGUGGUUCU	1342	1329-1351
AD-1700806.1	ACCACCCAGCCC AGAAGUUCU	895	1332-1352	AGAACUTCUGGGC UGGGUGGUUC	1343	1330-1352
AD-1700807.1	CCACCCAGCCCA GAAGUCCU	896	1333-1353	AGGAACTUCUGGG CUGGGUGGUU	1344	1331-1353

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700808.1	CACCCAGCCCAG AAGUUCCAU	897	1334-1354	AUGGAACUUCUG GGCUGGGUGGU	1345	1332-1354
AD-1700809.1	CCAGAAGUUCC AGGGAAGGAU	898	1342-1362	AUCCTUCCCUGGA ACUUCUGGGC	1346	1340-1362
AD-1700810.1	CAGAAGUUCCA GGGAAGGAAU	899	1343-1363	AUUCCUTCCCUGG AACUUCUGGG	1347	1341-1363
AD-1700811.1	AGAAGUUC CAG GGAAGGAACU	900	1344-1364	AGUUCCTUCCCUG GAACUUCUGG	1348	1342-1364
AD-1700812.1	GAAGUUC CAGG GAAGGAACUU	901	1345-1365	AAGUTCCUUCCCU GGAACUUCUG	1349	1343-1365
AD-1700813.1	AAGUUC CAGGG AAGGAACUCU	902	1346-1366	AGAGTUCCUUCCC UGGAACUUCU	1350	1344-1366
AD-1700814.1	AGUUC CAGGGA AGGAACUCUU	903	1347-1367	AAGAGUTCCUUC CUGGAACUUC	1351	1345-1367
AD-1700815.1	GUUC CAGGGAA GGAACUCUCU	904	1348-1368	AGAGAGTUCCUUC CCUGGAACUU	1352	1346-1368
AD-1700816.1	GUCCACCAUGG AGUACCUCUU	905	1370-1390	AAGAGGTACUCCA UGGUGGACCG	1353	1368-1390
AD-1700817.1	CACCAUGGAGU ACCUCUCAGU	906	1373-1393	ACUGAGAGGUAC UCCAUGGUGGA	1354	1371-1393
AD-1700819.1	CCAUGGAGUAC CUCUCAGCUU	907	1375-1395	AAGCTGAGAGGUA CUCCAUGGUG	1355	1373-1395
AD-1700821.1	AUGGAGUACCU CUCAGCUCUU	908	1377-1397	AAGAGCTGAGAGG UACUCCAUGG	1356	1375-1397
AD-1700822.1	UUACUCAGGUC AGUAUCUAAU	909	1410-1430	AUUAGATACUGAC CUGAGUAAU	1357	1408-1430
AD-1700824.1	UCAGUAUCUAA UAUAAGCUCU	910	1419-1439	AGAGCUTAUUAUA GAUACUGACC	1358	1417-1439
AD-1700825.1	CAGUAUCUAAU AUAAGCUCGU	911	1420-1440	ACGAGCTUAUAUU AGAUACUGAC	1359	1418-1440
AD-1700826.1	AGUAUCUAAUA UAAGCUCGGU	912	1421-1441	ACCGAGCUUAUAU UAGAUACUGA	1360	1419-1441
AD-1700828.1	UCUAAUAUAAG CUCGGAGUUU	913	1425-1445	AAACTCCGAGCUU AUUUUAGUA	1361	1423-1445
AD-1700829.1	CUAAUAUAAGC UCGGAGUUUU	914	1426-1446	AAAACUCCGAGCU UAUAUUAGAU	1362	1424-1446
AD-1700830.1	UAAGCUCGGAG UUUGGACGGU	915	1432-1452	ACCGTCCAAACUC CGAGCUUAUA	1363	1430-1452
AD-1700831.1	AAGCUCGGAGU UUGGACCGAU	916	1433-1453	AUCCGUCCAAACU CCGAGCUUAU	1364	1431-1453
AD-1700832.1	GGAGUUUGGAC GGAGGGUCUU	917	1439-1459	AAGACCCUCCGUC CAAACUCCGA	1365	1437-1459
AD-1700833.1	UGGACGGAGGG UCUGGACCUU	918	1445-1465	AAGGTCCAGACCC UCCGUCCAAA	1366	1443-1465
AD-1700834.1	AGCGACCUUUC GUGUCUGUU	919	1480-1500	AACAGACACGGAA AGGUCGCUGG	1367	1478-1500
AD-1700837.1	CAAGCGGACCA UCCGGAAGU	920	1505-1525	ACUUTCCGGAUGG UCCGCUUGUG	1368	1503-1525

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700838.1	CAUCCGGAAAGGCCUGACAGU	921	1514-1534	ACUGTCAGGCCUUUCCGGAUGGU	1369	1512-1534
AD-1700839.1	AGGAGCUGCUA GCCAAAGCAU	922	1546-1566	AUGCTUTGGCUAGCAGCUCCUGG	1370	1544-1566
AD-1700840.1	GGAGCUGCUAG CCAAAGCAUU	923	1547-1567	AAUGCUTUGGCUA GCAGCUCCUG	1371	1545-1567
AD-1700841.1	GAGCUGCUAGC CAAAGCAUUU	924	1548-1568	AAAUGCTUUGGCU AGCAGCUCCU	1372	1546-1568
AD-1700842.1	AGCUGCUAGCC AAAGCAUUGU	925	1549-1569	ACAATGCUUUGGC UAGCAGCUCC	1373	1547-1569
AD-1700843.1	CUAGCCAAAGC AUUGGAGACU	926	1554-1574	AGUCTCCAAUGCU UUGGCUAGCA	1374	1552-1574
AD-1700844.1	UAGCCAAAGCA UUGGAGACCU	927	1555-1575	AGGUCUCCAAUGC UUUGGCUAGC	1375	1553-1575
AD-1700845.1	AGCAUUGGAGA CCCUACUGCU	928	1562-1582	AGCAGUAGGGUC UCCAAUGCUUU	1376	1560-1582
AD-1700846.1	GCAUUGGAGAC CCUACUGCUU	929	1563-1583	AAGCAGTAGGGUC UCCAAUGCUU	1377	1561-1583
AD-1700848.1	AUUGGAGACCC UACUGCUGAU	930	1565-1585	AUCAGCAGUAGG GUCUCCAAUGC	1378	1563-1585
AD-1700850.1	GGAGACCCUAC UGCUGAAUGU	931	1568-1588	ACAUTCAGCAGUA GGGUCUCCAA	1379	1566-1588
AD-1700851.1	GAGACCCUACU GCUGAAUGGU	932	1569-1589	ACCATUCAGCAGU AGGGUCUCCA	1380	1567-1589
AD-1700852.1	ACCCUACUGCUG AAUGGAGUU	933	1572-1592	AACUCCAUUCAGC AGUAGGGUCU	1381	1570-1592
AD-1700853.1	CCCUACUGCUGA AUGGAGUGU	934	1573-1593	ACACTCCAUUCAG CAGUAGGGUC	1382	1571-1593
AD-1700854.1	CCUACUGCUGA AUGGAGUGCU	935	1574-1594	AGCACUCCAUAUCA GCAGUAGGGU	1383	1572-1594
AD-1700855.1	CUACUGCUGAA UGGAGUGCUU	936	1575-1595	AAGCACTCCAUUC AGCAGUAGGG	1384	1573-1595
AD-1700856.1	UACUGCUGAAU GGAGUGCUAU	937	1576-1596	AUAGCACUCCAUA CAGCAGUAGG	1385	1574-1596
AD-1700858.1	GCUGAAUGGAG UGCUAACCCU	938	1580-1600	AGGGTUAGCACUC CAUUCAGCAG	1386	1578-1600
AD-1700859.1	CUGAAUGGAGU GCUAACCCUU	939	1581-1601	AAGGGUTAGCACU CCAUUCAGCA	1387	1579-1601
AD-1700860.1	UGAAUGGAGUG CUAACCCUGU	940	1582-1602	ACAGGGTUAGCAC UCCAUAUCAGC	1388	1580-1602
AD-1700863.1	UGGAGUGCUAA CCCUGGUGCU	941	1586-1606	AGCACCAGGGUUA GCACUCCAUA	1389	1584-1606
AD-1700864.1	GGAGUGCUAAC CCUGGUGCUU	942	1587-1607	AAGCACCAGGGUU AGCACUCCAUA	1390	1585-1607
AD-1700865.1	GAGUGCUAACC CUGGUGCUAU	943	1588-1608	AUAGCACCAGGGU UAGCACUCCA	1391	1586-1608
AD-1700867.1	AACCCUGGUGC UAGAGGAGGU	944	1595-1615	ACCUCCTCUAGCA CCAGGGUUAG	1392	1593-1615

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700868.1	CCCUGGUGCUA GAGGAGGAUU	945	1597-1617	AAUCCUCCUCUAG CACCAGGGUU	1393	1595-1617
AD-1700869.1	GGUGCUAGAGG AGGAUGGAAU	946	1601-1621	AUUCCATCCUCCU CUAGCACCAG	1394	1599-1621
AD-1700870.1	GUGCUAGAGGA GGAUGGAACU	947	1602-1622	AGUUCCAUCCUCC UCUAGCACCA	1395	1600-1622
AD-1700871.1	CUAGAGGAGGA UGGAACUGCU	948	1605-1625	AGCAGUTCCAUCC UCCUCUAGCA	1396	1603-1625
AD-1700872.1	GAGGAUGGAAC UGCAGUGGAU	949	1611-1631	AUCCACTGCAGUU CCAUCCUCCU	1397	1609-1631
AD-1700873.1	GGAUGGAACUG CAGUGGACAU	950	1613-1633	AUGUCCACUGCAG UCCAUCCUC	1398	1611-1633
AD-1700874.1	GAUGGAACUGC AGUGGACAGU	951	1614-1634	ACUGTCCACUGCA GUCCAUCCU	1399	1612-1634
AD-1700875.1	AUGGAACUGCA GUGGACAGUU	952	1615-1635	AACUGUCCACUGC AGUCCAUCC	1400	1613-1635
AD-1700876.1	UGGAACUGCAG UGGACAGUGU	953	1616-1636	ACACTGTCCACUG CAGUCCAUC	1401	1614-1636
AD-1700877.1	GAACUGCAGUG GACAGUGAGU	954	1618-1638	ACUCACTGUCCAC UGCAGUCCA	1402	1616-1638
AD-1700878.1	ACUGCAGUGGA CAGUGAGGAU	955	1620-1640	AUCCTCACUGUCC ACUGCAGUUC	1403	1618-1640
AD-1700879.1	CUGCAGUGGAC AGUGAGGACU	956	1621-1641	AGUCCUCACUGUC CACUGCAGUU	1404	1619-1641
AD-1700880.1	UGCAGUGGACA GUGAGGACUU	957	1622-1642	AAGUCCTCACUGU CCACUGCAGU	1405	1620-1642
AD-1700881.1	GCAGUGGACAG UGAGGACUUU	958	1623-1643	AAAGTCCUCACUG UCCACUGCAG	1406	1621-1643
AD-1700882.1	CAGUGGACAGU GAGGACUUCU	959	1624-1644	AGAAGUCCUCACU GUCCACUGCA	1407	1622-1644
AD-1700883.1	AGUGGACAGUG AGGACUUCUU	960	1625-1645	AAGAAGTCCUCAC UGUCCACUGC	1408	1623-1645
AD-1700885.1	UGGACAGUGAG GACUUCUUCU	961	1627-1647	AGAAGAAGUCCUC ACUGUCCACU	1409	1625-1647
AD-1700887.1	CAGUGAGGACU UCUCCAGCU	962	1631-1651	AGCUGGAAGAAG UCCUCACUGUC	1410	1629-1651
AD-1700888.1	UGAGGACUUCU UCCAGCUGCU	963	1634-1654	AGCAGCTGGAAGA AGUCCUCACU	1411	1632-1654
AD-1700889.1	GAGGACUUCUU CCAGCUGCUU	964	1635-1655	AAGCAGCUGGAA GAAGUCCUCAC	1412	1633-1655
AD-1700891.1	GGACUUCUCC AGCUGCUGGU	965	1637-1657	ACCAGCAGCUGGA AGAAGUCCUC	1413	1635-1657
AD-1700892.1	UCUCCAGCUGC UGGAGGAUU	966	1642-1662	AAUCCUCCAGCAG CUGGAAGAAG	1414	1640-1662
AD-1700893.1	CAGCUGCUGGA GGAUGACACU	967	1647-1667	AGUGTCAUCCUCC AGCAGCUGGA	1415	1645-1667
AD-1700894.1	GCUGCUGGAGG AUGACACGUU	968	1649-1669	AACGTGTCAUCCU CCAGCAGCUG	1416	1647-1669

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700895.1	CUGGAGGAUGA CACGUGCCUU	969	1653-1673	AAGGCACGUGUCA UCCUCCAGCA	1417	1651-1673
AD-1700897.1	GGAUGACACGU GCCUGAUGGU	970	1658-1678	ACCATCAGGCACG UGUCAUCCUC	1418	1656-1678
AD-1700898.1	GAUGACACGUG CCUGAUGGUU	971	1659-1679	AACCAUCAGGCAC GUGUCAUCCU	1419	1657-1679
AD-1700899.1	GACACGUGCCU GAUGGUGUUU	972	1662-1682	AAACACCAUCAGG CACGUGUCAU	1420	1660-1682
AD-1700900.1	GCAGUCUGGUC AGAGCUGGAU	973	1682-1702	AUCCAGCUCUGAC CAGACUGCAA	1421	1680-1702
AD-1700902.1	UACAAGGAGUG GAGUGCUGUU	974	1706-1726	AACAGCACUCCAC UCCUUGUAGG	1422	1704-1726
AD-1700903.1	ACAAGGAGUGG AGUGCUGUCU	975	1707-1727	AGACAGCACUCCA CUCCUUGUAG	1423	1705-1727
AD-1700904.1	AAGGAGUGGAG UGCUGUCAUU	976	1709-1729	AAUGACAGCACUC CACUCCUUGU	1424	1707-1729
AD-1700905.1	GGAGUGGAGUG CUGUCAUAUU	977	1711-1731	AAUATGACAGCAC UCCACUCCUU	1425	1709-1731
AD-1700906.1	UGGAGUGCUGU CAUAUGGCCU	978	1715-1735	AGGCCATAUGACA GCACUCCACU	1426	1713-1735
AD-1700907.1	GGAGUGCUGUC AUAUGGCCUU	979	1716-1736	AAGGCCAUUAGAC AGCACUCCAC	1427	1714-1736
AD-1700908.1	GAGUGCUGUCA UAUGGCCUGU	980	1717-1737	ACAGGCCAUUAGA CAGCACUCCA	1428	1715-1737
AD-1700909.1	AGUGCUGUCAU AUGGCCUGGU	981	1718-1738	ACCAGGCCAUUAG ACAGCACUCC	1429	1716-1738
AD-1700912.1	GCUGUCAUAUG GCCUGGGACU	982	1721-1741	AGUCCCAGGCCAU AUGACAGCAC	1430	1719-1741
AD-1700913.1	CUGUCAUAUGG CCUGGGACGU	983	1722-1742	ACGUCCCAGGCCA UAUGACAGCA	1431	1720-1742
AD-1700915.1	CAAGCACAGCA AGGACAUCGU	984	1751-1771	ACGATGTCCUUGC UGUGCUUGGG	1432	1749-1771
AD-1700916.1	AUUCACCUUUG ACGUGUACAU	985	1775-1795	AUGUACACGUCAA AGGUGAAUCG	1433	1773-1795
AD-1700917.1	UUCACCUUUGA CGUGUACAAU	986	1776-1796	AUUGTACACGUCA AAGGUGAAUC	1434	1774-1796
AD-1700918.1	UCACCUUUGAC GUGUACAAGU	987	1777-1797	ACUUGUACACGUC AAAGGUGAAU	1435	1775-1797
AD-1700919.1	CACCUUUGACG UGUACAAGCU	988	1778-1798	AGCUTGTACACGU CAAAGGUGAA	1436	1776-1798
AD-1700920.1	CCUUUGACGUG UACAAGCAAU	989	1780-1800	AUUGCUTGUACAC GUCAAAGGUG	1437	1778-1800
AD-1700922.1	UGACGUGUACA AGCAAAACCU	990	1784-1804	AGGUTUTGCUUGU ACACGUCAAA	1438	1782-1804
AD-1700923.1	GACGUGUACAA GCAAAACCCU	991	1785-1805	AGGGTUTUGCUUG UACACGUCAA	1439	1783-1805
AD-1700924.1	ACGUGUACAAG CAAAACCCUU	992	1786-1806	AAGGGUTUUGCUU GUACACGUCA	1440	1784-1806

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700925.1	CGUGUACAAGC AAAACCCUCU	993	1787-1807	AGAGGGTUUUGCU UGUACACGUC	1441	1785-1807
AD-1700929.1	CAAGCAAAACCC UCGAGACCU	994	1793-1813	AGGUCUCGAGGG UUUUGCUUGUA	1442	1791-1813
AD-1700930.1	AAGCAAAACCC UCGAGACCU	995	1794-1814	AAGGTCTCGAGGG UUUUGCUUGU	1443	1792-1814
AD-1700931.1	AGCAAAACCCUC GAGACCUCU	996	1795-1815	AGAGGUCUCGAG GGUUUUGCUUG	1444	1793-1815
AD-1700934.1	GAGACCUCUUU GGCAGCCUGU	997	1807-1827	ACAGGCTGCCAAA GAGGUCUCGA	1445	1805-1827
AD-1700936.1	CCUCUUUGGCA GCCUGAAUGU	998	1811-1831	ACAUTCAGGCUGC CAAAGAGGUC	1446	1809-1831
AD-1700937.1	CUCUUUGGCAG CCUGAAUGUU	999	1812-1832	AACATUCAGGCUG CCAAAGAGGU	1447	1810-1832
AD-1700938.1	CUUUGGCAGCC UGAAUGUCAU	1000	1814-1834	AUGACATUCAGGC UGCCAAAGAG	1448	1812-1834
AD-1700939.1	UUUGGCAGCCU GAAUGUCAAU	1001	1815-1835	AUUGACAUUCAG GCUGCCAAAGA	1449	1813-1835
AD-1700940.1	UGGCAGCCUGA AUGUCAAAAGU	1002	1817-1837	ACUUTGACAUUCA GGCUGCCAAA	1450	1815-1837
AD-1700942.1	GCAGCCUGAAU GUCAAAGCCU	1003	1819-1839	AGGCTUTGACAUU CAGGCUGCCA	1451	1817-1839
AD-1700943.1	CAGCCUGAAUG UCAAGCCAU	1004	1820-1840	AUGGCUTUGACAU UCAGGCUGCC	1452	1818-1840
AD-1700944.1	AGCCUGAAUGU CAAAGCCACU	1005	1821-1841	AGUGGCTUUGACA UUCAGGCUGC	1453	1819-1841
AD-1700947.1	AUGUCAAAAGCC ACAUUCUACU	1006	1828-1848	AGUAGAAUGUGG CUUUGACAUUC	1454	1826-1848
AD-1700948.1	AGCCACAUUCU ACGGGCUCUU	1007	1835-1855	AAGAGCCCGUAGA AUGUGGCUUU	1455	1833-1855
AD-1700949.1	GCCACAUUCUAC GGGCUCUAU	1008	1836-1856	AUAGAGCCCGUAG AAUGUGGCUU	1456	1834-1856
AD-1700950.1	CACAUUCUACG GGCUCUACUU	1009	1838-1858	AAGUAGAGCCCGU AGAAUGUGGC	1457	1836-1858
AD-1700952.1	CAUUCUACGGG CUCUACUCUU	1010	1840-1860	AAGAGUAGAGCCC GUAGAAUGUG	1458	1838-1860
AD-1700953.1	AUUCUACGGGC UCUACUCUAU	1011	1841-1861	AUAGAGTAGAGCC CGUAGAAUGU	1459	1839-1861
AD-1700955.1	UCUACGGGCUC UACUCUAUGU	1012	1843-1863	ACAUAGAGUAGA GCCCGUAGAAU	1460	1841-1863
AD-1700957.1	ACGGGCUCUAC UCUAUGAGUU	1013	1846-1866	AACUCATAGAGUA GAGCCCGUAG	1461	1844-1866
AD-1700958.1	GGGCUCUACUC UAUGAGUUGU	1014	1848-1868	ACAACUCAUAGAG UAGAGCCCGU	1462	1846-1868
AD-1700959.1	GGCUCUACUCU AUGAGUUGUU	1015	1849-1869	AACAACUCAUAGA GUAGAGCCCG	1463	1847-1869
AD-1700960.1	CUACUCUAUGA GUUGUGACUU	1016	1853-1873	AAGUCACAACUCA UAGAGUAGAG	1464	1851-1873

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700961.1	UACUCUAUGAGUUGUGACUUU	1017	1854-1874	AAAGTCACAACUCAUAGAGUAGA	1465	1852-1874
AD-1700962.1	ACUCUAUGAGUUGUGACUUUU	1018	1855-1875	AAAAGUCACAACUCAUAGAGUAG	1466	1853-1875
AD-1700963.1	CUCUAUGAGUUGUGACUUUCU	1019	1856-1876	AGAAAGTCACAACUCAUAGAGUA	1467	1854-1876
AD-1700965.1	CUAUGAGUUGUGACUUUCAU	1020	1858-1878	AUUGAAAGUCACAACUCAUAGAG	1468	1856-1878
AD-1700967.1	GAGUUGUGACUUUCAAGGACU	1021	1862-1882	AGUCCUTGAAAGUCACAACUCAU	1469	1860-1882
AD-1700968.1	AGUUGUGACUUUCAAGGACUU	1022	1863-1883	AAGUCCTUGAAAGUCACAACUCA	1470	1861-1883
AD-1700969.1	UUGGCCCAAAGAAAGUACUCU	1023	1882-1902	AGAGTACUUUCUUUGGCCCAAGU	1471	1880-1902
AD-1700970.1	UGGCCCAAAGAAAGUACUCAU	1024	1883-1903	AUGAGUACUUUCUUUGGCCCAAG	1472	1881-1903
AD-1700971.1	GGCCCAAAGAAAGUACUCAGU	1025	1884-1904	ACUGAGTACUUUCUUUGGCCCAA	1473	1882-1904
AD-1700973.1	CCCAAAGAAAGUACUCAGGGU	1026	1886-1906	ACCCTGAGUACUUUCUUUGGCC	1474	1884-1906
AD-1700975.1	AAAGAAAGUACUCAGGGAGCU	1027	1889-1909	AGCUCCCUGAGUACUUUCUUUGG	1475	1887-1909
AD-1700976.1	AAGAAAGUACUCAGGGAGCUU	1028	1890-1910	AAGCTCCCUGAGUACUUUCUUUG	1476	1888-1910
AD-1700977.1	AGAAAGUACUCAGGGAGCUCU	1029	1891-1911	AGAGCUCCCUGAGUACUUUCUUU	1477	1889-1911
AD-1700978.1	AGUACUCAGGGAGCUCCUUCU	1030	1895-1915	AGAAGGAGCUCCCUGAGUACUUU	1478	1893-1915
AD-1700980.1	AGGGAGCUCCUUCGUUGGACU	1031	1902-1922	AGUCCAACGAAGGAGCUCCCUGA	1479	1900-1922
AD-1700981.1	GGAGCUCCUUCGUUGGACCUU	1032	1904-1924	AAGGTCCAACGAAGGAGCUCCCU	1480	1902-1924
AD-1700982.1	UUCGUUGGACCUCACACUGU	1033	1912-1932	ACAGTGTGGAGGUCCAACGAAGG	1481	1910-1932
AD-1700984.1	CGUUGGACCUCACACUGCUU	1034	1914-1934	AAGCAGTGUGGAGGUCCAACGA	1482	1912-1934
AD-1700985.1	GACCUCCACACUGCUGCAAGU	1035	1919-1939	ACUUGCAGCAGUGUGGAGGUCCA	1483	1917-1939
AD-1700986.1	GGCCUGGGCCAUAUGUUGCUU	1036	1938-1958	AAGCAACAUAUGGCCAGGCCUU	1484	1936-1958
AD-1700988.1	GGGCCAUAUGUUGCUGGGAAU	1037	1943-1963	AUUCCCAGCAACAUAUGGCCAG	1485	1941-1963
AD-1700989.1	GGCCAUAUGUUGCUGGGAAUU	1038	1944-1964	AAUCCCAGCAACAUAUGGCCCA	1486	1942-1964
AD-1700990.1	CCAUAUGUUGCUGGGAAUUUU	1039	1946-1966	AAAATUCCCAGCAACAUAUGGCC	1487	1944-1966
AD-1700991.1	CAUAUGUUGCUGGGAAUUUCU	1040	1947-1967	AGAAAUTCCCAGCAACAUAUGGC	1488	1945-1967

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1700992.1	UGUUGCUGGGA AUUCCUCCU	1041	1951-1971	AGGAGGAAAUUC CCAGCAACAU	1489	1949-1971
AD-1700995.1	UGGGAAUUUCC UCCACCCUUU	1042	1957-1977	AAAGGGTGGAGG AAAUUCCCAGC	1490	1955-1977
AD-1700997.1	UCCUCCACCCU UCGUCAUGU	1043	1964-1984	ACAUGACGAAGG GUGGAGGAAAU	1491	1962-1984
AD-1700998.1	AAGGGCCGCCUC CAUCCUAU	1044	2013-2033	AUAGGAAUGGAG GCGGCCCUUCU	1492	2011-2033
AD-1701001.1	GCCGCCUCCAUU CCUACUAAU	1045	2017-2037	AUUAGUAGGAAU GGAGGCGGCCC	1493	2015-2037
AD-1701002.1	CCGCCUCCAUUC CUACUAAGU	1046	2018-2038	ACUUAGTAGGAU GGAGGCGGCC	1494	2016-2038
AD-1701003.1	CCCACUGCAAAG ACUAUGACU	1047	2076-2096	AGUCAUAGUCUU UGCAGUGGGUC	1495	2074-2096
AD-1701004.1	CCACUGCAAAG ACUAUGACAU	1048	2077-2097	AUGUCATAGUCUU UGCAGUGGGU	1496	2075-2097
AD-1701005.1	CACUGCAAAGA CUAUGACAGU	1049	2078-2098	ACUGTCAUAGUCU UUGCAGUGGG	1497	2076-2098
AD-1701007.1	AAGACUAUGAC AGCAUCAAU	1050	2085-2105	AUUUGATGCUGUC AUAGUCUUUG	1498	2083-2105
AD-1701009.1	GACAGCAUCAA AUUUCAGGAU	1051	2093-2113	AUCCTGAAAUUUG AUGCUGUCAU	1499	2091-2113
AD-1701011.1	CAGCAUCAAU UUCAGGACCU	1052	2095-2115	AGGUCCTGAAAUU UGAUGCUGUC	1500	2093-2115
AD-1701012.1	AGCAUCAAU UCAGGACCUU	1053	2096-2116	AAGGTCCUGAAU UUGAUGCUGU	1501	2094-2116
AD-1701013.1	GCAUCAAUUU CAGGACCUGU	1054	2097-2117	ACAGGUCCUGAAA UUUGAUGCUG	1502	2095-2117
AD-1701014.1	CAUCAAUUUC AGGACCUGCU	1055	2098-2118	AGCAGGTCCUGAA AUUUGAUGCUG	1503	2096-2118
AD-1701016.1	AUUUCAGGACC UGCAGACAGU	1056	2104-2124	ACUGTCTGCAGGU CCUGAAAUUU	1504	2102-2124
AD-1701017.1	UUUCAGGACCU GCAGACAGUU	1057	2105-2125	AACUGUCUGCAGG UCCUGAAAUU	1505	2103-2125
AD-1701019.1	CAGGACCUGCA GACAGUACAU	1058	2108-2128	AUGUACTGUCUGC AGGUCCUGAA	1506	2106-2128
AD-1701020.1	GACCUGCAGAC AGUACAGGCU	1059	2111-2131	AGCCTGTACUGUC UGCAGGUCCU	1507	2109-2131
AD-1701022.1	CCUGCAGACAG UACAGGCUAU	1060	2113-2133	AUAGCCTGUACUG UCUGCAGGUC	1508	2111-2133
AD-1701023.1	CUGCAGACAGU ACAGGCUAGU	1061	2114-2134	ACUAGCCUGUACU GUCUGCAGGU	1509	2112-2134
AD-1701024.1	UGCAGACAGUA CAGGCUAGAU	1062	2115-2135	AUCUAGCCUGUAC UGUCUGCAGG	1510	2113-2135
AD-1701026.1	CAGACAGUACA GGCUAGAUU	1063	2117-2137	AUAUCUAGCCUGU ACUGUCUGCA	1511	2115-2137
AD-1701027.1	GACAGUACAGG CUAGAUAAU	1064	2119-2139	AGUUAUCUAGCCU GUACUGUCUG	1512	2117-2139

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1701029.1	CCUAGCCACCUU UCUGGCCUU	1065	2243-2263	AAGGCCAGAAAG GUGGCUAGGAA	1513	2241-2263
AD-1701031.1	CUUUCUGGCCUC CCACUUGCU	1066	2252-2272	AGCAAGTGGGAGG CCAGAAAGGU	1514	2250-2272
AD-1701032.1	UCUGGCCUCCCA CUUGCCCUU	1067	2255-2275	AAGGGCAAGUGG GAGGCCAGAAA	1515	2253-2275
AD-1701033.1	GGCAUCUUGAU CCCAGCCUGU	1068	2302-2322	ACAGGCTGGGAUC AAGAUGCCUG	1516	2300-2322
AD-1701034.1	GCAUCUUGAUC CCAGCCUGAU	1069	2303-2323	AUCAGGCUGGGA UCAAGAUGCCU	1517	2301-2323
AD-1701035.1	ACCAAUGCCUCC UGUCCCUAU	1070	2345-2365	AUAGGGACAGGA GGCAUUGGUAG	1518	2343-2365
AD-1701037.1	AUGCCUCCUGUC CCUAAACUU	1071	2349-2369	AAGUTUAGGGACA GGAGGCAUUG	1519	2347-2369
AD-1701038.1	GCCUCCUGUCCC UAAACUCCU	1072	2351-2371	AGGAGUTUAGGG ACAGGAGGCAU	1520	2349-2371
AD-1720280.1	AAAACCCUCGA GACCUCUUUU	1073	1798-1818	AAAAGAGGUCUC GAGGGUUUUGC	1521	1796-1818
AD-1720281.1	CUGGGCUGAGA UCCCAGGUUU	1074	1272-1292	AAACCTGGGAUCU CAGCCCAGGA	1522	1270-1292
AD-1720282.1	GUGGACAGUGA GGACUUCUUU	1075	1626-1646	AAAGAAGUCCUCA CUGUCCACUG	1523	1624-1646
AD-1720283.1	CAAAACCCUCGA GACCUCUUU	1076	1797-1817	AAAGAGGTCUCGA GGGUUUUGCU	1524	1795-1817
AD-1720284.1	CUGAAUGUCAA AGCCACAUUU	1077	1824-1844	AAATGTGGCUUTG ACAUUCAGGC	1525	1822-1844
AD-1720285.1	GGCUCUACUCU AUGAGUUGUU	1078	1849-1869	AACAACCTCAUAGA GUAGAGCCCG	1526	1847-1869
AD-1720286.1	AAUGGAGUGCU AACCUGGUU	1079	1584-1604	AACCAGGGUUAGC ACUCCAUUCA	1527	1582-1604
AD-1720287.1	AAUGGAGUGCU AACCUUGGUU	1080	1584-1604	AACCAAGGUUAGC ACUCCAUUCA	1528	1582-1604
AD-1720288.1	AAUGGAGUGCU AACUCUGGUU	1081	1584-1604	AACCAGAGUUAGC ACUCCAUUCA	1529	1582-1604
AD-1720289.1	ACCCUACUGCUG AAUGGAGUU	1082	1572-1592	AACUCCAUUCAGC AGUAGGGUCU	1530	1570-1592
AD-1720290.1	ACCUGCAGACA GUACAGGCUU	1083	2112-2132	AAGCCTGUACUGU CUGCAGGUCC	1531	2110-2132
AD-1720291.1	CUGGGAAUUUC CUCCACCCUU	1084	1956-1976	AAGGGTGGAGGA AAUUCCAGCA	1532	1954-1976
AD-1720292.1	GGGCCGCCUCCA UUCCUACUU	1085	2015-2035	AAGUAGGAAUGG AGGCGGCCCUU	1533	2013-2035
AD-1720293.1	UUCUACGGGCU CUACUCUAUU	1086	1842-1862	AAUAGAGUAGAG CCCGUAGAAUG	1534	1840-1862
AD-1720294.1	GCAGACAGUAC AGGCUAGAUU	1087	2116-2136	AAUCUAGCCUGTA CUGUCUGCAG	1535	2114-2136
AD-1720295.1	UGGAGACCCUA CUGCUGAAUU	1088	1567-1587	AAUTCAGCAGUAG GGUCUCCAAU	1536	1565-1587

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1720296.1	ACCUCUUUGGC AGCCUGAAUU	1089	1810-1830	AAUTCAGGCUGCC AAAGAGGUCU	1537	1808-1830
AD-1720297.1	CCUUUCUGGCCU CCCACUUGU	1090	2251-2271	ACAAGTGGGAGGC CAGAAAGGUG	1538	2249-2271
AD-1720298.1	UGGAACUGCAG UGGACAGUGU	1091	1616-1636	ACACTGTCCACTG CAGUUCCAUC	1539	1614-1636
AD-1720299.1	AGGAUGACACG UGCCUGAUGU	1092	1657-1677	ACATCAGGCACGU GUCAUCCUCC	1540	1655-1677
AD-1720300.1	GAAUGGAGUGC UAACCCUGGU	1093	1583-1603	ACCAGGGTUAGCA CUCCAUUCAG	1541	1581-1603
AD-1720301.1	GUGCUGUCAUA UGGCCUGGGU	1094	1719-1739	ACCCAGGCCAUUA GACAGCACUC	1542	1717-1739
AD-1720302.1	GUGCUGUCAUA UGGCUUGGGU	1095	1719-1739	ACCCAAGCCAUAU GACAGCACUC	1543	1717-1739
AD-1720303.1	GUGCUGUCAUA UGGUCUGGGU	1096	1719-1739	ACCCAGACCAUAU GACAGCACUC	1544	1717-1739
AD-1720304.1	GCCCAAAGAAA GUACUCAGGU	1097	1885-1905	ACCTGAGUACUTU CUUUGGGCCA	1545	1883-1905
AD-1720305.1	GUACUCAGGGA GCUCCUUCGU	1098	1896-1916	ACGAAGGAGCUCC CUGAGUACUU	1546	1894-1916
AD-1720306.1	GGAUUUCCUC CACCCUUCGU	1099	1959-1979	ACGAAGGGUGGA GGAAAUUCCCA	1547	1957-1979
AD-1720307.1	GGAUUUCCUC CACCCUUCGU	1100	1959-1979	ACGAAAGGUGGA GGAAAUUCCCA	1548	1957-1979
AD-1720308.1	GGAUUUCCUC CACUCUUCGU	1101	1959-1979	ACGAAGAGUGGA GGAAAUUCCCA	1549	1957-1979
AD-1720309.1	GUGUACAAGCA AAACCCUCGU	1102	1788-1808	ACGAGGGTUUUGC UUGUACACGU	1550	1786-1808
AD-1720310.1	CAGUCUGGUCA GAGCUGGAGU	1103	1683-1703	ACUCCAGCUCUGA CCAGACUGCA	1551	1681-1703
AD-1720311.1	UGCUAACCCUG GUGCUAGAGU	1104	1591-1611	ACUCUAGCACCAG GGUUAGCACU	1552	1589-1611
AD-1720312.1	UCAAUUUCAG GACCUGCAGU	1105	2100-2120	ACUGCAGGUCCTG AAAUUUGAUG	1553	2098-2120
AD-1720313.1	AGAACCACCCAG CCCAGAAGU	1106	1329-1349	ACUTCTGGGCUGG GUGGUUCUCU	1554	1327-1349
AD-1720314.1	CAAACUAACAA AAACAUUUCU	1107	2434-2454	AGAAATGUUUUTG UUAGUUUGAG	1555	2432-2454
AD-1720315.1	CAUGGAGUACC UCUCAGCUCU	1108	1376-1396	AGAGCTGAGAGGU ACUCCAUGGU	1556	1374-1396
AD-1720316.1	UCGUUGGACCU CCACACUGCU	1109	1913-1933	AGCAGTGUGGAGG UCCAACGAAG	1557	1911-1933
AD-1720317.1	ACCAUGGAGUA CCUCUCAGCU	1110	1374-1394	AGCTGAGAGGUAC UCCAUGGUGG	1558	1372-1394
AD-1720368.1	GGCAGCCUGAA UGUCAAGCU	1111	1818-1838	AGCTUTGACAUTC AGGCUGCCAA	1559	1816-1838
AD-1720369.1	UCCUAGCCACCU UUCUGGCCU	1112	2242-2262	AGGCCAGAAAGG UGGCUAGGAAA	1560	2240-2262

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in NM_001393338.1
AD-1720370.1	UGCUGAAUGGA GUGCUAACCU	1113	1579-1599	AGGTUAGCACUCC AUUCAGCAGU	1561	1577-1599
AD-1720371.1	UCAGGACCUGC AGACAGUACU	1114	2107-2127	AGUACTGUCUGCA GGUCCUGAAA	1562	2105-2127
AD-1720372.1	ACAGCAUCAAA UUUCAGGACU	1115	2094-2114	AGUCCTGAAAUTU GAUGCUGUCA	1563	2092-2114
AD-1720373.1	UUGCUGGGAAU UUCUCCACU	1116	1953-1973	AGUGGAGGAAAT UCCAGCAACA	1564	1951-1973
AD-1720374.1	AAUGCCUCCUG UCCCUAAACU	1117	2348-2368	AGUTUAGGGACAG GAGGCAUUGG	1565	2346-2368
AD-1720375.1	UUGACGUGUAC AAGCAAAACU	1118	1783-1803	AGUTUTGCUUGTA CACGUCAAAG	1566	1781-1803
AD-1720376.1	GGCCGCCUCCAU UCCUACUAU	1119	2016-2036	ATAGUAGGAAUG GAGGCGGCCCU	1567	2014-2036
AD-1720377.1	ACUCAGGUCAG UAUCUAAUAU	1120	1412-1432	ATATUAGAUACTG ACCUGAGUAA	1568	1410-1432
AD-1720378.1	UGGGCCAUAUG UUGCUGGGAU	1121	1942-1962	ATCCCAGCAACAU AUGGCCCAGG	1569	1940-1962
AD-1720379.1	UGCUGUCAUAU GGCCUGGGAU	1122	1720-1740	ATCCCAGGCCATA UGACAGCACU	1570	1718-1740
AD-1720380.1	UGAGUUGUGAC UUUCAAGGAU	1123	1861-1881	ATCCUTGAAAGTC ACAACUCAUA	1571	1859-1881
AD-1720381.1	AGGCCCAAGCAC AGCAAGGAU	1124	1746-1766	ATCCUTGCUGUGC UUGGGCCUCU	1572	1744-1766
AD-1720382.1	UGUACAAGCAA AACCUCGAU	1125	1789-1809	ATCGAGGGUUTG CUUGUACACG	1573	1787-1809
AD-1720383.1	UGUACAAGCAA AACCUUCGAU	1126	1789-1809	ATCGAAGGUUTG CUUGUACACG	1574	1787-1809
AD-1720384.1	UGUACAAGCAA AACUCUCGAU	1127	1789-1809	ATCGAGAGUUTG CUUGUACACG	1575	1787-1809
AD-1720385.1	UCUAUGAGUUG UGACUUUCAU	1128	1857-1877	ATGAAAGUCACAA CUCAUAGAGU	1576	1855-1877
AD-1720386.1	AAAGACUAUGA CAGCAUCAAU	1129	2084-2104	ATUGATGCUGUCA UAGUCUUUGC	1577	2082-2104

Table 6. Modified Sense and Antisense Strand Sequences of Human CIDEB dsRNA Agents

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1699864.1	gsusccugggCfUfGf agaucccaguL96	1578	asdCsugdGgdAucucdAg Cfccaggacsasa	2026	GGAAGGAAAGAGCA GAUCCCAGG	2474
AD-1699867.1	csusgggcugAfGfAf ucccagguuuL96	1579	asdAsacdCudGggaudCu Cfagcccagsgsa	2027	AGGAAAGAGCAGAU CCCAGGUUU	2475
AD-1699868.1	usgsggcugaGfAfUf cccagguuuuL96	1580	asdAsaadCcdTgggadTcU fcagcccagsgsg	2028	GGAAAGAGCAGAUCC CCAGGUUUG	2476
AD-1699869.1	gsgsgcugagAfUfCf ccagguuuuL96	1581	asdCsaadAcdCugggdAu Cfucagcccsasg	2029	GAAAGAGCAGAUCC CAGGUUUGU	2477

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1699870.1	gsgscugagaUfCfCf cagguuuguuL96	1582	asdAscAdAadCcuggdGa Ufcucagccscsa	2030	AAAGAGCAGAUCCC AGGUUUGUA	2478
AD-1699871.1	gscsugagauCfCfCf agguuuguauL96	1583	asdTsacdAadAccugdGgA fucucagcscsc	2031	AAGAGCAGAUCCCA GGUUUGUAA	2479
AD-1699872.1	csusgagauCfCfAf gguuuguauL96	1584	asdTsuadCadAaccudGgG faucucagcscsc	2032	AGAGCAGAUCCCAG GUUUGUAAAC	2480
AD-1699873.1	usgsagauccCfAfGf guuuguauL96	1585	asdGsuudAcAaacedTgG fgaucucagsgsc	2033	GAGCAGAUCCCAGG UUUGUAAACA	2481
AD-1699875.1	asgsaucccaGfGfUf uuguuacaguL96	1586	asdCsugdTudAcaaadCcU fgggaucuscscsa	2034	GCAGAUCCCAGGUU UGUAAACAGA	2482
AD-1699882.1	asgsguuuguAfAfCf agaaaacacuL96	1587	asdGsugdTudTucugdTuA fcaaacucsgsg	2035	CCAGGUUUGUAAACA GAAAACACC	2483
AD-1699891.1	ascsgaaaaCfAfCfc acuaaagcuL96	1588	asdGscudTudAguggdGg Ufuucucugususa	2036	UAACAGAAAACACC ACUAAAGCC	2484
AD-1699892.1	csasgaaaacAfCfCfa cuaaaagccuL96	1589	asdGsgcdTudTagugdGu Gfuuuucugsusu	2037	AACAGAAAACACCA CUAAAGCCC	2485
AD-1699904.1	asgsaaccacCfCfAfg cccagaaguL96	1590	asdCsuudCudGggcudGg Gfugguucuscscu	2038	AGAGAACCACCCAG CCCAGAAGU	2486
AD-1699907.1	ascscaccaGfCfCfc agaaguucuL96	1591	asdGsaadCudTcuggdGcU fggguggususc	2039	GAACCACCCAGCCC AGAAGUUC	2487
AD-1699948.1	csasccauggAfGfUf accucucaguL96	1592	asdCsugdAgdAgguadCu Cfcuuggugsgsa	2040	UCCACCAUGGAGUA CCUCUCAGC	2488
AD-1699949.1	ascscaugaGfUfAf ccucucaguL96	1593	asdGscudGadGaggudAc Ufccuuggugsgsg	2041	CCACCAUGGAGUAC CUCUCAGCU	2489
AD-1699951.1	csasuggaguAfCfCf ucucagcucuL96	1594	asdGsagdCudGagagdGu Afccuagsgsgsu	2042	ACCAUGGAGUACCU CUCAGCUCU	2490
AD-1699964.1	csusuacucaGfGfUf caguaucuaL96	1595	asdTsagdAudAcugadCcU fgaguaagsusc	2043	GACUUACUCAGGUC AGUAUCUAA	2491
AD-1699967.1	ascscagguCfAfGf uaucuaauL96	1596	asdTsaudTadGauacdTgA fccugagusasa	2044	UUACUCAGGUCAGU AUCUAAUAU	2492
AD-1699970.1	csasggucagUfAfUf cuaauauauL96	1597	asdTsuadTadTuagadTaCf ugaccugsasg	2045	CUCAGGUCAGUAUC UAAUAUAAG	2493
AD-1699971.1	asgsgucaguAfUfCf uaauauaaguL96	1598	asdCsuudAudAuagadAu Afccugaccusgsa	2046	UCAGGUCAGUAUCU AAUAUAAGC	2494
AD-1699972.1	gsgscagauUfCfUf aaauaaagcuL96	1599	asdGscudTadTauuadGaU facugaccusg	2047	CAGGUCAGUAUCUA AUUAAGCU	2495
AD-1699973.1	gsuscagauCfUfAf auauaagcuL96	1600	asdAsgedTudAuauudAg Afuacugacscsu	2048	AGGUCAGUAUCUAA UAUAAGCUC	2496
AD-1699976.1	asgsuaucaAfUfAf uaagcucggL96	1601	asdCscgdAgdCuauadAu Ufagauacusgsa	2049	UCAGUAUCUAAUAU AAGCUCGGA	2497
AD-1699977.1	gsusaucauUfAfUf aagcucggauL96	1602	asdTsccdGadGcuuadTaU fuagauacsug	2050	CAGUAUCUAAUAUA AGCUCGGAG	2498
AD-1699978.1	usasucuaaAfUfAf agcucggaguL96	1603	asdCsucdCgdAgcuudAu Afuuagauascsu	2051	AGUAUCUAAUAUA AAGCUCGGAGU	2499
AD-1699979.1	asuscuaauUfAfAf gcucggaguL96	1604	asdAscudCcdGagcudTaU fauuagausc	2052	GUAUCUAAUAUAAG CUCGGAGUU	2500
AD-1699982.1	usasauaaaGfCfUf cggaguuguL96	1605	asdCsaadAcdTccgadGcU fuauauuasgsa	2053	UCUAAUAUAAGCUC GGAGUUUGG	2501

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1699983.1	asasuauaagCfUfCf ggaguuugguL96	1606	asdCscadAadCuccgdAgC fuuauauusasg	2054	CUAAUUAAGCUCG GAGUUUGGA	2502
AD-1699984.1	asusauaagcUfCfGf gaguuuggauL96	1607	asdTscadAadAcuccdGaG fcuuauaususa	2055	UAAUUAAGCUCGG AGUUUGGAC	2503
AD-1699985.1	usasuaagcuCfGfGf aguuuggacuL96	1608	asdGsuedCadAacucdCgA fgcuuauasusu	2056	AAUUAAGCUCGGA GUUUGGACG	2504
AD-1699986.1	asusaagcucGfGfAf guuuggacguL96	1609	asdCsgudCcdAaacudCcG fagcuuauasusu	2057	AUAUAAGCUCGGAG UUUGGACGG	2505
AD-1699987.1	usasagcucgGfAfGf uuuggacgguL96	1610	asdCscgdTcdCaaacdTcCf gagcuuauasusa	2058	UAUAAGCUCGGAGU UUGGACGGA	2506
AD-1699988.1	asasgcucggAfGfUf uuggacggauL96	1611	asdTscadGudCcaaadCuC fcgagcuuauasusu	2059	AUAAGCUCGGAGUU UGGACGGAG	2507
AD-1699989.1	asgscucggaGfUfUf uggacggaguL96	1612	asdCsuedCgdTccaadAcU fccgagcususa	2060	UAAGCUCGGAGUUU GGACGGAGG	2508
AD-1699990.1	gscscgagGfUfUf ggacggagguL96	1613	asdCscudCcdGuccadAaC fuccgagcsusu	2061	AAGCUCGGAGUUUG GACGGAGGG	2509
AD-1700015.1	asgscgaccuUfUfCf cgugucuguuL96	1614	asdAscadGadCacggdAaA fggucgcsusg	2062	CCAGCGACCUUCC GUGUCUGUG	2510
AD-1700035.1	gsasucacaaGfCfGf gaccauccguL96	1615	asdCsggdAudGguccdGc Ufugugaucsc	2063	GUGAUCACAAGCGG ACCAUCCGG	2511
AD-1700037.1	uscsacaagcGfGfAf ccauccggauL96	1616	asdTscadGgdAuggdCc Gfcuugugasusc	2064	GAUCACAAGCGGAC CAUCCGGAA	2512
AD-1700038.1	csascaagcgGfAfCfc auccggauL96	1617	asdTsuedCgdGauggdTcC fgcuuuggsasu	2065	AUCACAAGCGGACC AUCCGGAAA	2513
AD-1700039.1	ascsaagcggAfCfCfa uccggaaauL96	1618	asdTsuudCcdGgaugdGu Cfcgcuugusgsa	2066	UCACAAGCGGACCA UCCGGAAAG	2514
AD-1700040.1	csasagcggcCfCfAf uccggaaaguL96	1619	asdCsuidTcdCggauGg Ufccgcuugsusg	2067	CACAAGCGGACCAU CCGGAAAGG	2515
AD-1700041.1	asasgcggacCfAfUf ccggaaagguL96	1620	asdCscudTudCcgadTgG fuccgcuusgsu	2068	ACAAGCGGACCAUC CGGAAAGGC	2516
AD-1700077.1	gsgsagcugcUfAfGf ccaaagcauuL96	1621	asdAsugdCudTuggcdTaG fcagcuusgsu	2069	CAGGAGCUGCUAGC CAAAGCAUU	2517
AD-1700080.1	gscsugcuagCfCfAf aagcauugguL96	1622	asdCscadAudGcuuudGg Cfuagcagsusc	2070	GAGCUGCUAGCCAA AGCAUUGGA	2518
AD-1700081.1	csuscuagcCfAfAf agcauuggauL96	1623	asdTscadAadTgcuudTgG fcuagcagscsu	2071	AGCUGCUAGCCAAA GCAUUGGAG	2519
AD-1700082.1	usgscuagccAfAfAf gcuauggaguL96	1624	asdCsuedCadAugcdTuG fgcuaagcagcsc	2072	GCUGCUAGCCAAAG CAUUGGAGA	2520
AD-1700083.1	gscsuagccaAfAfGf cauuggagauL96	1625	asdTscudCcdAaugcdTuU fggcuagcsasg	2073	CUGCUAGCCAAAGC AUUGGAGAC	2521
AD-1700091.1	asasgcauugGfAfGf acccuacuguL96	1626	asdCsagdTadGggucdTcC faaugcuusug	2074	CAAAGCAUUGGAGA CCCUACUGC	2522
AD-1700092.1	asgscuuggAfGfAf cccuacugcuL96	1627	asdGscadGudAgggudCu Cfcaaugcsusu	2075	AAAGCAUUGGAGAC CCUACUGCU	2523
AD-1700094.1	csasuuggagAfCfCf cuacugcuguL96	1628	asdCsagdCadGuaggdGu Cfuccaugsusu	2076	AGCAUUGGAGACCC UACUGCUGA	2524
AD-1700097.1	usgsgagaccCfUfAf cugcugaaauL96	1629	asdAsuudCadGcagudAg Gfgucuccasasu	2077	AUUGGAGACCCUAC UGCUGAAUG	2525

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700099.1	gsasgaccuAfCfUf gcugaauugguL96	1630	asdCscadTudCagcadGuA fgggucuscscsa	2078	UGGAGACCCUACUG CUGAAUGGA	2526
AD-1700100.1	asgsaccuaCfUfGfc ugaauuggauL96	1631	asdTscadAudTcagcdAgU faggucuscsc	2079	GGAGACCCUACUGC UGAAUGGAG	2527
AD-1700101.1	gsascccuacUfGfCf ugaauuggaguL96	1632	asdCsudCadTucagdCaG fuaggucuscsc	2080	GAGACCCUACUGCU GAAUGGAGU	2528
AD-1700104.1	cscsuacugcUfGfAf auggagugcuL96	1633	asdGscadCudCcuaudCaG fcaguaggsgsu	2081	ACCCUACUGCUGAA UGGAGUGCU	2529
AD-1700109.1	usgscugaauGfGfAf gugcuaaccuL96	1634	asdGsgudTadGcacudCcA fuucagcasgsu	2082	ACUGCUGAAUGGAG UGCUAACCC	2530
AD-1700112.1	usgsaauggaGfUfGf cuaaccugcuL96	1635	asdCsagdGgdTuagcdAcU fccauucascsc	2083	GCUGAAUGGAGUGC UAACCCUGG	2531
AD-1700113.1	gsasauggagUfGfCf uaaccugguL96	1636	asdCscadGgdGuuagdCaC fuccauucscasg	2084	CUGAAUGGAGUGCU AACCCUGGU	2532
AD-1700114.1	asasuggaguGfCfUf aaccugguuL96	1637	asdAscCdAgdGguuadGc Afucceauscsa	2085	UGAAUGGAGUGCUA ACCCUGGUG	2533
AD-1700115.1	asusggagugCfUfAf acccugguuL96	1638	asdCsacdCadGgguudAg Cfacuccaususc	2086	GAAUGGAGUGCUAA CCCUGGUGC	2534
AD-1700116.1	usgsgagugcUfAfAf cccugguuL96	1639	asdGscadCcdAgggudTaG fccuccasusu	2087	AAUGGAGUGCUAAC CCUGGUGCU	2535
AD-1700121.1	usgscuaaccCfUfGf gugcuagaguL96	1640	asdCsudTadGcaccdAgG fguuagcascsu	2088	AGUGCUAACCCUGG UGCUAGAGG	2536
AD-1700122.1	gsesuaaccUfGfGf ugcuaagaguL96	1641	asdCsudCudAgcacdCaG fgguuagcsasc	2089	GUGCUAACCCUGGU GCUAGAGGA	2537
AD-1700125.1	asasccugugUfGfCf uagaggagguL96	1642	asdCsudCcdTcuagdCaC fcaggguuasag	2090	CUAACCCUGGUGCU AGAGGAGGA	2538
AD-1700148.1	gsasacugcaGfUfGf gacagugaguL96	1643	asdCsudAcdTguccdAcU fgcaguucscsa	2091	UGGAACUGCAGUGG ACAGUGAGG	2539
AD-1700149.1	asascugcagUfGfGf acagugaguL96	1644	asdCsudCadCugucdCaC fugcaguuscsc	2092	GGAACUGCAGUGGA CAGUGAGGA	2540
AD-1700156.1	gsusggacagUfGfAf ggacuucuuuL96	1645	asdAsagdAadGuccudCaC fuguccacsusg	2093	CAGUGGACAGUGAG GACUUCUUC	2541
AD-1700158.1	gsgsacagugAfGfGf acuucuuuL96	1646	asdGsgadAgdAagucdCu Cfacuguccsasc	2094	GUGGACAGUGAGGA CUUCUCCA	2542
AD-1700159.1	gsascagugaGfGfAf cuucuuuccauL96	1647	asdTsggdAadGaagudCcU fccagucscsa	2095	UGGACAGUGAGGAC UUCUCCAG	2543
AD-1700160.1	asesagugagGfAfCf uuucuuuccaguL96	1648	asdCsugdGadAgaagdTcC fucacuguscsc	2096	GGACAGUGAGGACU UCUCCAGC	2544
AD-1700166.1	asgsgacuucUfUfCf cagcugcuguL96	1649	asdCsagdCadGcuggdAa Gfaaguccscsa	2097	UGAGGACUUCUCC AGCUGCUGG	2545
AD-1700167.1	gsgsacuucUfCfCf agcugcugguL96	1650	asdCscadGcdAgcugdGa Afgaaguccsusc	2098	GAGGACUUCUCCA GCUGCUGGA	2546
AD-1700180.1	csusgcuggaGfGfAf ugacacguguL96	1651	asdCsacdGudGucaudCcU fccagcagcsu	2099	AGCUGCUGGAGGAU GACACGUGC	2547
AD-1700187.1	asgsgaugacAfCfGf ugccugauguL96	1652	asdCsaudCadGgcacdGuG fucauccuscsc	2100	GGAGGAUGACACGU GCCUGAUGG	2548
AD-1700188.1	gsgsaugacaCfGfUf gccugaugguL96	1653	asdCscadTcdAggcadCgU fgucuccsusc	2101	GAGGAUGACACGUG CCUGAUGGU	2549

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700189.1	gsasugacacGfUfGf ccugaugguuL96	1654	asdAsccdAudCaggcdAc Gfugucaucscsu	2102	AGGAUGACACGUGC CUGAUGGUG	2550
AD-1700190.1	asusgacacgUfGfCf cugaugguuL96	1655	asdCsacdCadTcaggdCaC fgugucauscsc	2103	GGAUGACACGUGCC UGAUGGUGU	2551
AD-1700191.1	usgsacacguGfCfCf ugaugguuL96	1656	asdAscadCcdAucagdGcA fcgugucasusc	2104	GAUGACACGUGCCU GAUGGUGUU	2552
AD-1700193.1	ascacgugcCfUfGf augguuuL96	1657	asdCsaadCadCcaucdAgG fcacguguscsa	2105	UGACACGUGCCUGA UGGUGUUGC	2553
AD-1700213.1	csasgucuggUfCfAf gagcuggaguL96	1658	asdCsucdCadGcucudGaC fcagacugscsa	2106	UGCAGUCUGGUCAG AGCUGGAGC	2554
AD-1700242.1	gsasguggagUfGfCf ugucauauL96	1659	asdCsaudAudGacagdCaC fuccacucscsu	2107	AGGAGUGGAGUGCU GUCAUAUGG	2555
AD-1700243.1	asgsuggaguGfCfUf gucauauL96	1660	asdCscadTadTgacadGcA fcuccacuscsc	2108	GGAGUGGAGUGCUG UCAUAUGGC	2556
AD-1700244.1	gsusggagugCfUfGf ucauauL96	1661	asdGscddAudAugacdAg Cfacuccacsusc	2109	GAGUGGAGUGCUGU CAUAUGGCC	2557
AD-1700245.1	usgsagugcUfGfUf cauauL96	1662	asdGsgcdCadTaugadCaG fcacuccacsu	2110	AGUGGAGUGCUGUC AUAUGGCCU	2558
AD-1700248.1	asgsugcuguCfAfUf auggccuL96	1663	asdCscadGgdCcauadTgA fcagcacuscsc	2111	GGAGUGCUGUCAUA UGGCCUGGG	2559
AD-1700249.1	gsusgcugucAfUfAf uggccuL96	1664	asdCscddAgdGccaudAu Gfacagcacsusc	2112	GAGUGCUGUCAUAU GGCCUGGGA	2560
AD-1700250.1	usgscugucaUfAfUf ggccuL96	1665	asdTscddCadGgccadTaU fgacagcscsu	2113	AGUGCUGUCAUAUG GCCUGGGAC	2561
AD-1700252.1	csusgucauaUfGfGf ccugggacguL96	1666	asdCsgudCcdCaggcdCaU faugacagscsa	2114	UGCUGUCAUAUGGC CUGGGACGG	2562
AD-1700272.1	asgsgcccaaGfCfAfc agcaaggauL96	1667	asdTscddTudGcugudGcU fugggccuscscsu	2115	AGAGGCCCAAGCAC AGCAAGGAC	2563
AD-1700279.1	asgscacagcAfAfGf gacaucgcuL96	1668	asdGsgcdGadTguccdTuG fcugugcusug	2116	CAAGCACAGCAAGG ACAUCGCCC	2564
AD-1700280.1	gsesacagcaAfGfGf acaucgcccL96	1669	asdGsggdCgdAugudCu Ufgcugugcsusu	2117	AAGCACAGCAAGGA CAUCGCCCCG	2565
AD-1700303.1	uscsaccuuuGfAfCf guguaaaguL96	1670	asdCsudGudAcacgdTcA faaggugasasu	2118	AUUCACCUUUGACG UGUACAAGC	2566
AD-1700309.1	usugacgugUfAfCf aagcaaaacuL96	1671	asdGsuudTudGcuugdTaC facgucaasasg	2119	CUUUGACGUGUACA AGCAAAACC	2567
AD-1700312.1	asesguguacAfAfGf caaaaccuuL96	1672	asdAsggdGudTuugcdTu Gfuacacguscsa	2120	UGACGUGUACAAGC AAAACCCUC	2568
AD-1700314.1	gsusguacaaGfCfAf aaaccucguL96	1673	asdCsgadGgdGuuudGc Ufuguacacsgsu	2121	ACGUGUACAAGCAA AACCUCGA	2569
AD-1700315.1	usgsuacaagCfAfAf aaccucgauL96	1674	asdTscgdAgdGguuudTg Cfuuguacascsg	2122	CGUGUACAAGCAAA ACCCUCGAG	2570
AD-1700316.1	gsusacaagcAfAfAf acccucgaguL96	1675	asdCsucdGadGgguudTu Gfcuuguacsasc	2123	GUGUACAAGCAAAA CCCUCGAGA	2571
AD-1700317.1	usascaagcaAfAfAf cccucgagauL96	1676	asdTscudCgdAgggudTu Ufgcuuguascsa	2124	UGUACAAGCAAAAC CCUCGAGAC	2572
AD-1700318.1	ascsaagcaaAfAfCfc cucgagacuL96	1677	asdGsucdTcdGagggdTuU fugcuugusasc	2125	GUACAAGCAAAACC CUCGAGACC	2573

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700323.1	csasaaacccUfCfGfa gaccucuuuL96	1678	asdAsagdAgdGucudGa Gfgguuuugscsu	2126	AGCAAAACCCUCGA GACCUCUUU	2574
AD-1700324.1	asasaacccuCfGfAfg accucuuuuL96	1679	asdAsaadGadGgucudCg Afgguuuuugsc	2127	GCAAAACCCUCGAG ACCUCUUUG	2575
AD-1700325.1	asasaccucGfAfGfa ccucuuuguL96	1680	asdCsaadAgdAggucdTcG faggguuusug	2128	CAAAACCCUCGAGA CCUCUUUGG	2576
AD-1700327.1	ascscucgaGfAfCfc ucuuuggcuL96	1681	asdGscdAadAgaggdTcU fcgaggguusuu	2129	AAACCCUCGAGACC UCUUUGGCA	2577
AD-1700328.1	cscscucgagAfCfCf ucuuuggcauL96	1682	asdTsgcdCadAagagdGuC fucgagggsuu	2130	AACCCUCGAGACCU CUUUGGCAG	2578
AD-1700329.1	cscsucgagaCfCfUfc uuuggcaguL96	1683	asdCsugdCcdAaagadGg Ufcucgaggsgsu	2131	ACCCUCGAGACCUC UUUGGCAGC	2579
AD-1700336.1	ascscucuuuGfGfCf agccugaaauL96	1684	asdAsuudCadGgcugdCc Afaagagguscsu	2132	AGACCUCUUUGGCA GCCUGAUG	2580
AD-1700339.1	uscuuuuggcAfGfCf cugaaugucuL96	1685	asdGsacdAudTcaggdCuG fccaaagasgsg	2133	CCUCUUUGGCAGCC UGAAUGUCA	2581
AD-1700344.1	gsgscagccuGfAfAf ugucaaauguL96	1686	asdGscudTudGacaudTcA fggcugccsasa	2134	UUGGCAGCCUGAAU GUCAAAGCC	2582
AD-1700350.1	csusgaauGuCfAfAf agccacauuuL96	1687	asdAsaudGudGgcudTg Afcuuucagsgsc	2135	GCCUGAAUGUCAA GCCACAUUC	2583
AD-1700352.1	gsasaugucaAfAfGf ccacauucuuL96	1688	asdAsgadAudGuggdTcU Ufgacauucsasg	2136	CUGAAUGUCAAAGC CACAUUCUA	2584
AD-1700353.1	asasugucAAfGfCf cacauucuaL96	1689	asdTsagdAadTgaggdCuU fugacauuscsa	2137	UGAAUGUCAAAGCC ACAUUCUAC	2585
AD-1700354.1	asusgucaaaGfCfCfa cauucuaL96	1690	asdGsuadGadAugudGc Ufuugacaususc	2138	GAAUGUCAAAGCCA CAUUCUACG	2586
AD-1700355.1	usgsucaaaGfCfAf cauucuaL96	1691	asdCsgudAgdAaugudGg Cfuugacausuu	2139	AAUGUCAAAGCCAC AUUCUACGG	2587
AD-1700356.1	gsuscaaaGfCfCfa uucuaL96	1692	asdCscgdTadGaaugdTgG fcuuugacsasu	2140	AUGUCAAAGCCACA UUCUACGGG	2588
AD-1700357.1	uscscagccAfCfAf uucuaL96	1693	asdCscdGudAgaudGu Gfgcuuugascsa	2141	UGUCAAAGCCACA UCUACGGGC	2589
AD-1700358.1	csasaagccaCfAfUfu cuacgggcuL96	1694	asdGscdCgdTagaadTgU fggcuuugsasc	2142	GUCAAAGCCACA CUACGGGCU	2590
AD-1700359.1	asasagccAcfUfUfc uacgggcuL96	1695	asdAsgcdCcdGuagadAu Gfuggcuuusgsa	2143	UCAAGCCACA UACGGGCUC	2591
AD-1700365.1	asesauucuaCfGfGf gcucuaL96	1696	asdGsagdTadGagcdCgU fagaugusgsg	2144	CCACAUUCUACGGG CUCUACUCU	2592
AD-1700366.1	csasuucuaGfGfGf cucuaL96	1697	asdAsgadGudAgagcdCc Gfuagaugsusg	2145	CACAUUCUACGGGC UCUACUCUA	2593
AD-1700368.1	ususcuaaggGfCfUf cuacucuaL96	1698	asdAsuadGadGuagadGc Cfcguagaasusg	2146	CAUUCUACGGGCUC UACUCUAUG	2594
AD-1700369.1	uscscuacggGfUfCf uacucuaL96	1699	asdCsaudAgdAguagadAg Cfccguagasasu	2147	AUUCUACGGGCUCU ACUCUAUGA	2595
AD-1700370.1	csusacgggcUfCfUf acucuaL96	1700	asdTscadTadGaguadGaG fccguagsasa	2148	UUCUACGGGCUCUA CUCUAUGAG	2596
AD-1700371.1	usascgggcUfUfAf cucuaL96	1701	asdCsudAudAgagudAg Afgccguagsasa	2149	UCUACGGGCUCUAC UCUAUGAGU	2597

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700372.1	ascsgggcucUfAfCf ucuauagauuL96	1702	asdAscudCadTagagdTaG fagcccgusasg	2150	CUACGGGCUCUACU CUAUGAGUU	2598
AD-1700374.1	gsgsgcucuaCfUfCf uauagauuguL96	1703	asdCsaadCudCauagdAgU fagagcccsusu	2151	ACGGGCUCUACUCU AUGAGUUGU	2599
AD-1700375.1	gsgscucuacUfCfUf augaguuguuL96	1704	asdAscadAcdTcauadGaG fuagagccscsg	2152	CGGGCUCUACUCUA UGAGUUGUG	2600
AD-1700376.1	gscsucuacuCfUfAf ugaguuguuL96	1705	asdCsacdAadCucaudAgA fguagagcscsc	2153	GGGCUCUACUCUAU GAGUUGUGA	2601
AD-1700378.1	uscsuacucuAfUfGf aguugugacuL96	1706	asdGsucdAcdAacudAu Afgaguagsgsc	2154	GCUCUACUCUAUGA GUUGUGACU	2602
AD-1700383.1	uscsuauagagUfUfGf ugacuuucauL96	1707	asdTsgadAadGucacdAaC fucauagagsu	2155	ACUCUAUGAGUUGU GACUUUCA	2603
AD-1700384.1	csusaugaguUfGfUf gacuuucaauL96	1708	asdTsugdAadAgucadCaA fcucauagsasg	2156	CUCUAUGAGUUGUG ACUUUCAAG	2604
AD-1700385.1	usasugaguUfUfGf acuuucaaguL96	1709	asdCsuudGadAagucdAc Afacucauasgsa	2157	UCUAUGAGUUGUGA CUUUCAAGG	2605
AD-1700386.1	asusgaguugUfGfAf cuuucagguL96	1710	asdCscudTgdAaagudCaC faacucausasg	2158	CUAUGAGUUGUGAC UUUCAAGGA	2606
AD-1700387.1	usgsaguuguGfAfCf uuucaaggauL96	1711	asdTscddTudGaaagdTcA fcaacucasusa	2159	UAUGAGUUGUGACU UUCAAGGAC	2607
AD-1700388.1	gsasguugugAfCfUf uucaaggacuL96	1712	asdGsucdCudTgaaadGuC facaacucsasu	2160	AUGAGUUGUGACUU UCAAGGACU	2608
AD-1700408.1	ususggcccaAfAfGf aaaguacucuL96	1713	asdGsagdTadCuuucdTuU fgggccaasgsu	2161	ACUUGGCCCAAAGA AAGUACUCA	2609
AD-1700410.1	gsgscccaaaGfAfAf aguacucaguL96	1714	asdCsugdAgdTacuudTcU fuugggcsasa	2162	UUGGCCCAAAGAAA GUACUCAGG	2610
AD-1700411.1	gscsccaaagAfAfAf guacucagguL96	1715	asdCscudGadGuacudTuC fuuugggcsasa	2163	UGGCCCAAAGAAAG UACUCAGGG	2611
AD-1700413.1	cscsaaagaaAfGfUfa cucagggauL96	1716	asdTscddCudGaguadCuU fucuuuggsgsc	2164	GCCCAAAGAAAGUA CUCAGGGAG	2612
AD-1700414.1	csasaagaaaGfUfAfc ucagggaguL96	1717	asdCsucdCcdTgagudAcU fuucuuuggsgsg	2165	CCCAAAGAAAGUAC UCAGGGAGC	2613
AD-1700422.1	gsusacucagGfGfAf gcuccuucguL96	1718	asdCsgadAgdGagcudCcC fugaguacsusu	2166	AAGUACUCAGGGAG CUCCUUCGU	2614
AD-1700423.1	usascucaggGfAfGf cuccuucguuL96	1719	asdAscgdAadGgagedTcC fcugaguascsu	2167	AGUACUCAGGGAGC UCCUUCGUU	2615
AD-1700424.1	ascsucaggGfGfCf uccuucguuuL96	1720	asdAsacdGadAggagdCu Cfccugagusasc	2168	GUACUCAGGGAGCU CCUUCGUUG	2616
AD-1700425.1	csuscagggaGfCfUf ccuucguuguL96	1721	asdCsaadCgdAaggadGcU fccugagsusa	2169	UACUCAGGGAGCUC CUUCGUUGG	2617
AD-1700426.1	uscsaggagCfUfCf cuucguugguL96	1722	asdCscadAcdGaaggdAgC fuccugagsu	2170	ACUCAGGGAGCUCC UUCGUUGGA	2618
AD-1700427.1	csasgggagCfCfCf uucguuggauL96	1723	asdTscddAadCgaagdGaG fcuccugsasg	2171	CUCAGGGAGCUCCU UCGUUGGAC	2619
AD-1700428.1	asgsggagcuCfCfUf ucguuggacuL96	1724	asdGsucdCadAcgaadGg Afgcuccusgsa	2172	UCAGGGAGCUCCUU CGUUGGACC	2620
AD-1700439.1	uscsuuggaCfCfUf ccacacugcuL96	1725	asdGscadGudGuggadGg Ufccacgasasg	2173	CUUCGUUGGACCUC CACACUGCU	2621

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700461.1	csasaggccuGfGfGf ccuauuguuuL96	1726	asdAsacdAudAuggcdCc Afggccuugscsa	2174	UGCAAGGCCUGGGC CAUAUGUUG	2622
AD-1700462.1	asasggccugGfGfCf cauauuguuL96	1727	asdCsaadCadTauggdCcC faggccuugsc	2175	GCAAGGCCUGGGCC AUAUGUUGC	2623
AD-1700464.1	gsgscugggCfCfAf uauuguucuuL96	1728	asdAsgcdAadCauaudGg Cfccaggccsusu	2176	AAGGCCUGGGCCAU AUGUUGCUG	2624
AD-1700465.1	gscscugggCfAfUf auguugcuguL96	1729	asdCsagdCadAcaudTgG fcccaggccscsu	2177	AGGCCUGGGCCAU UGUUGCUGG	2625
AD-1700468.1	usgsggccauAfUfGf uugcugggauL96	1730	asdTscddCadGcaacdAuA fuggcccasgsg	2178	CCUGGGCCAU AUGU UGCUGGGAA	2626
AD-1700473.1	csasauuguuGfCfUf gggauuuuL96	1731	asdGsaadAudTcccadGcA facauaugsgsc	2179	GCCAU AUGUUGCUG GGAAUUUCC	2627
AD-1700474.1	asusauguuGfUfGf ggaauuuuL96	1732	asdGsgadAadTucccdAgC faacauaugsgsg	2180	CCAU AUGUUGCUGG GAAUUUCCU	2628
AD-1700476.1	asusguugcuGfGfGf aauuuccucuL96	1733	asdGsagdGadAauucdCc Afgcaacausasu	2181	AUAUGUUGCUGGGA AUUCCUCC	2629
AD-1700477.1	usgsuugcuGfGfAf auuuccuccuL96	1734	asdGsgadGgdAaaudCc Cfagcaacasusa	2182	UAUGUUGCUGGGAA UUCCUCCA	2630
AD-1700479.1	ususcugggAfAfUf uuccuccacuL96	1735	asdGsugdGadGgaadTuC fccagcaascsa	2183	UGUUGCUGGGAAU UCCUCCACC	2631
AD-1700482.1	csusgggaauUfUfCf cuccaccuuL96	1736	asdAsggdGudGgaggdAa Afuucccagscsa	2184	UGCUGGGAAUUUCC UCCACCCU	2632
AD-1700485.1	gsgsaauuucCfUfCf caccuucguL96	1737	asdCsgadAgdGguggdAg Gfaaauuccscsa	2185	UGGGAAUUUCCUCC ACCCUUCGU	2633
AD-1700486.1	gsasauuuccUfCfCf acccuucguuL96	1738	asdAscgdAadGggugdGa Gfgaaauucscsc	2186	GGGAAUUUCCUCCA CCCUUCGUC	2634
AD-1700487.1	asasuuccuCfCfAf cccuucgucuL96	1739	asdGsacdGadAgggudGg Afggaaauucscsc	2187	GGAAUUUCCUCCAC CCUUCGUCA	2635
AD-1700488.1	asusuuccuCfAfCf ccuucgucuL96	1740	asdTsgadCgdAagggdTgG faggaaususc	2188	GAAUUUCCUCCACC CUUCGUCAU	2636
AD-1700489.1	ususuccuccAfCfCf cuucgucuauL96	1741	asdAsugdAcdGaaggdGu Gfgaggaaasusu	2189	AAUUUCCUCCACCC UUCGUCAUG	2637
AD-1700490.1	ususcuccaCfCfCfu ucgucauguL96	1742	asdCsaudGadCgaagdGg Ufgagggaasasu	2190	AUUUCCUCCACCCU UCGUCAUGC	2638
AD-1700517.1	asasgggcccCfCfUf ccuauuccuauL96	1743	asdTsagdGadAuggadGg Cfggcccuuscsu	2191	AGAAGGGCCGCCUC CAUCCUAC	2639
AD-1700519.1	gsgsgcccccUfCfCf auuccuacuuL96	1744	asdAsgudAgdGaaugdGa Gfgcgcccsusu	2192	AAGGGCCGCCUCCA UCCUACUA	2640
AD-1700520.1	gsgscgcccCfCfAf uuccuacuuL96	1745	asdTsagdTadGgaudGgA fggcgcccsusu	2193	AGGGCCGCCUCCA UCCUACUAA	2641
AD-1700522.1	cscsgccuccAfUfUf ccuacuaaguL96	1746	asdCsaudAgdTaggadAu Gfgaggcgsgscsc	2194	GGCCGCCUCCAUC CUACUAAGG	2642
AD-1700542.1	cscscacugcAfAfAf gacuaugacuL96	1747	asdGsucdAudAgucudTu Gfcagugggsusc	2195	GACCCACUGCAAAG ACUAUGACA	2643
AD-1700544.1	csascugcaaAfGfAf cuaugacaguL96	1748	asdCsugdTcdAuagudCu Ufugcagugsgsg	2196	CCCACUGCAAAGAC UAUGACAGC	2644
AD-1700550.1	asasagacuaUfGfAf cagcaucaauL96	1749	asdTsugdAudGcugudCa Ufagucuuusgsc	2197	GCAAAGACUAUGAC AGCAUCAA	2645

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700553.1	gsascuaugaCfAfGfcaucaaauuuL96	1750	asdAsaudTudGaugcdTgUfcuagucsusu	2198	AAGACUAUGACAGCAUCAAUUUU	2646
AD-1700554.1	ascsuauagacAfGfCfaucaaauuuL96	1751	asdAsaadTudTgaugdCuGfucuauguscsu	2199	AGACUAUGACAGCAUCAAUUUC	2647
AD-1700555.1	csusaugacaGfCfAfucaaaauuucL96	1752	asdGsaadAudTugaudGcUfgucuaugsusc	2200	GACUAUGACAGCAUCAAUUUCA	2648
AD-1700556.1	usasugacagCfAfUfcaaaauuucL96	1753	asdTsgadAadTuugadTgCfugucuaasgsu	2201	ACUAUGACAGCAUCAAUUUCAG	2649
AD-1700557.1	asusgacagcAfUfCfaaaauucaguL96	1754	asdCsugdAadAuuugdAuGfcugucausasg	2202	CUAUGACAGCAUCAAAUUUCAGG	2650
AD-1700558.1	usgsacagcaUfCfAfaaaauucagguL96	1755	asdCscudGadAaaauudGaUfgucugucasusa	2203	UAUGACAGCAUCAAUUUCAGGA	2651
AD-1700560.1	ascsgagcaucAfAfAfaaaauucaggacuL96	1756	asdGsuedCudGaaudTuGfaugcuguscsa	2204	UGACAGCAUCAAUUUCAGGACC	2652
AD-1700563.1	gsesaucaaaUfUfUfcaggaccuguL96	1757	asdCsagdGudCcugadAaUfuugaugsusg	2205	CAGCAUCAAUUUCAGGACCUGC	2653
AD-1700566.1	uscsaaaauuUfCfAfgaccugcaguL96	1758	asdCsugdCadGgucddTgAfaaaauugasusg	2206	CAUCAAUUUCAGGACCUGCAGA	2654
AD-1700573.1	uscsaggaccUfGfCfagacaguacuL96	1759	asdGsuaadCudGucugdCaGfguccugasasa	2207	UUUCAGGACCUGCAGACAGUACA	2655
AD-1700575.1	asgsgaccugCfAfGfagacaguacuL96	1760	asdCsugdTadCugucddTgCfagguccusgsa	2208	UCAGGACCUGCAGACAGUACAGG	2656
AD-1700576.1	gsgsaccugcAfGfAfcaguacagguL96	1761	asdCscudGudAcugudCuGfcagguccsusg	2209	CAGGACCUGCAGACAGUACAGGC	2657
AD-1700578.1	ascscugcagAfCfAfguacagguuL96	1762	asdAsgcdCudGuacudGuCfugcagguscsc	2210	GGACCUGCAGACAGUACAGGCUA	2658
AD-1700580.1	csusgcagacAfGfUfacaggcuaguL96	1763	asdCsuaadGcdCuguadCuGfucugcagsgsu	2211	ACCUGCAGACAGUACAGGCUAGA	2659
AD-1700582.1	gsesagacagUfAfCfaggcuagauL96	1764	asdAsuedTadGccugdTaCfugucugcsasg	2212	CUGCAGACAGUACAGGCUAGUA	2660
AD-1700623.1	ascsgccucaCfAfUfccaagucuuL96	1765	asdAsgadCudTgggadTgUfgaggcgususa	2213	UAACGCCUCACAUCCCAAGUCUA	2661
AD-1700670.1	ususccuagcCfAfCfcuuucugguuL96	1766	asdGscddAgdAaaggdTgGfcuaggaasasg	2214	CUUCCUAGCCACC UUUCUGGCC	2662
AD-1700671.1	uscsuagccAfCfCfuuuucuggccuL96	1767	asdGsgcdCadGaaagdGuGfgcuaggasasa	2215	UUCCUAGCCACC UUUCUGGCCU	2663
AD-1700680.1	csesuucugGfCfCfuuccacuuguL96	1768	asdCsaadGudGggagdGcCfagaaggsusg	2216	CACCUUUCUGGCCU CCCACUUGC	2664
AD-1700681.1	csusuucuggCfCfUfcccacuugcuL96	1769	asdGscadAgdTgggadGgCfcagaagsgsu	2217	ACCUUUCUGGCCU CCACUUGCC	2665
AD-1700684.1	usesuggccuCfCfCfacuugcccuL96	1770	asdAsggdGcdAagugdGgAfggcccagasa	2218	UUUCUGGCCUCCCUUUGCCCUG	2666
AD-1700710.1	gsgscaucuuGfAfUfcccagccuguL96	1771	asdCsagdGcdTgggadTcAfagaugccsusg	2219	CAGGCAUCUUGAUC CCAGCCUGA	2667
AD-1700736.1	asasugccucCfUfGfuucccuaaacuL96	1772	asdGsuaadTadGggacdAgGfaggcauusgsg	2220	CCAAUGCCUCCUGU CCCUAAACU	2668
AD-1700737.1	asusgccuccUfGfUfcccuaaacuuL96	1773	asdAsgudTudAgggadCaGfgaggcausug	2221	CAAUGCCUCCUGU CCUAAACUC	2669

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700738.1	usgscuccuGfUfCf ccuaaacucuL96	1774	asdGsagdTudTagggdAcA fggaggcasusu	2222	AAUGCCUCCUGUCC CUAACUCC	2670
AD-1700780.1	csasaacuaaCfAfAfa aacauuucuL96	1775	asdGsaadAudGuuuudTg Ufuaguuuugsasg	2223	CUCAAACUAACAAA ACAUUUCC	2671
AD-1700781.1	asasacuaacAfAfAfa acauuuccuL96	1776	asdGsgadAadTguuudTu Gfuaguuuusgsa	2224	UCAAACUAACAAAA ACAUUCCA	2672
AD-1700782.1	asascuaacaAfAfAfa cauuccauL96	1777	asdTsggdAadAuguudTu Ufguuaguusug	2225	CAAACUAACAAAA CAUUUCCAA	2673
AD-1700791.1	gsuscugGfgCfUfG fagauccaguL96	1778	asCfsugdGg(Agn)ucucag CfcCfaggacsasa	2226	GGAAGGAAAGAGCA GAUCCAGG	2674
AD-1700793.1	usgsagauCfcCfAfG fguuuguaacuL96	1779	asGfsuudAc(Agn)aaccug GfgAfucucasgsc	2227	GAGCAGAUCCCAGG UUUGUAACA	2675
AD-1700794.1	gsasgaucCfcAfGfG fuuuguaacauL96	1780	asUfsgudTa(Cgn)aaaccu GfgGfaucucasg	2228	AGCAGAUCCCAGGU UUGUAACAG	2676
AD-1700795.1	gsasucccAfgGfUfU fuguaacagauL96	1781	asUfscudGu(Tgn)acaaac CfuGfggaucsusc	2229	CAGAUCCCAGGUUU GUAACAGAA	2677
AD-1700796.1	cscsagguUfuGfUfA facagaaaacuL96	1782	asGfsuudTu(Cgn)uguuac AfaAfcucggsa	2230	UCCCAGGUUUGUAA CAGAAAACA	2678
AD-1700797.1	csasgguUfgUfAf AfacagaaacauL96	1783	asUfsgudTu(Tgn)cuguua CfaAfaccugsgsg	2231	CCCAGGUUUGUAAC AGAAAACAC	2679
AD-1700798.1	asgsguuUfuAfAf CfagaaacacuL96	1784	asGfsugdTu(Tgn)ucuguu AfcAfaaccusgsg	2232	CCAGGUUUGUAACA GAAAACACC	2680
AD-1700799.1	gsgsuuugUfaAfCf AfgaaaacacuL96	1785	asGfsugdGu(Tgn)uucugu UfaCfaaaccusg	2233	CAGGUUUGUAACAG AAAACACCA	2681
AD-1700800.1	usasacagAfaAfAfC faccacuaaaL96	1786	asUfsuudAg(Tgn)gguguu UfuCfuguuascsa	2234	UGUAACAGAAAACA CCACUAAAG	2682
AD-1700801.1	csasgaaaAfcAfCfC facuaagccuL96	1787	asGfsgcdTu(Tgn)aguggu GfuUfuucugsusu	2235	AACAGAAAACACCA CUAAGCCC	2683
AD-1700802.1	csascaggAfgAfGfA faccaccaguL96	1788	asCfsugdGg(Tgn)gguuu CfuCfcugugscsu	2236	AGCACAGGAGAGAA CCACCCAGC	2684
AD-1700804.1	gsasaccaCfcCfAfG fcccagaaguL96	1789	asAfsudTc(Tgn)gggcu GfgUfgguucsusc	2237	GAGAACCACCCAGC CCAGAAGUU	2685
AD-1700805.1	asascacCfcAfGfCf ccagaaguL96	1790	asAfsadTu(Cgn)ugggu CfgGfugguucsu	2238	AGAACCACCCAGCC CAGAAGUUC	2686
AD-1700806.1	ascscaccCfaGfCfCf cagaaguL96	1791	asGfsaadCu(Tgn)cugggc UfgGfguggususc	2239	GAACCACCCAGCCC AGAAGUCC	2687
AD-1700807.1	cscsaccAfgCfCfCf agaaguL96	1792	asGfsgadAc(Tgn)ucugg CfuGfggugsusu	2240	AACCACCCAGCCCA GAAGUCCA	2688
AD-1700808.1	csascccaGfcCfCfAf gaaguL96	1793	asUfsggdAa(Cgn)uucugg GfcUfgggugsgsu	2241	ACCACCCAGCCCAG AAGUCCAG	2689
AD-1700809.1	cscsagaaGfuUfCfC faggaaggauL96	1794	asUfscdTu(Cgn)ccugga AfcUfucugsgsc	2242	GCCAGAAGUCCA GGAAGGAA	2690
AD-1700810.1	csasgaagUfuCfCfA fgggaaggauL96	1795	asUfsudCu(Tgn)cccugg AfaCfuucugsgsg	2243	CCCAGAAGUCCAG GGAAGAAC	2691
AD-1700811.1	asgsaaguUfcCfAfG fgaaggaacuL96	1796	asGfsuudCc(Tgn)uccug GfaAfcuucsgsg	2244	CCAGAAGUCCAGG GAAGGAACU	2692
AD-1700812.1	gsasaguuCfcAfGfG fgaaggaacuL96	1797	asAfsudTc(Cgn)uuccu GfgAfacuucsgsg	2245	CAGAAGUCCAGGG AAGGAACUC	2693

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700813.1	asasguucCfaGfGfGfaaggaacucuL96	1798	asGfsagdTu(Cgn)cuuccUfgGfaacuuscsu	2246	AGAAGUUCCAGGGAAGGAACUCU	2694
AD-1700814.1	asgsuuccAfgGfGfAfaggaacucuuL96	1799	asAfsgadGu(Tgn)ccuuccCfuGfgaacususc	2247	GAAGUUCCAGGGGAAAGGAACUCUC	2695
AD-1700815.1	gsusuccaGfgGfAfAfaggaacucucuL96	1800	asGfsagdAg(Tgn)uccuucCfcUfggaacsusu	2248	AAGUUCCAGGGAAGGAACUCUCC	2696
AD-1700816.1	gsusccacCfaUfGfGfaguaccucuuL96	1801	asAfsgadGg(Tgn)acuccaUfgGfuggacscsg	2249	CGGUCCACCAUGGAGUACCUCUC	2697
AD-1700817.1	csasccauGfgAfGfUfaccucucaguL96	1802	asCfsugdAg(Agn)gguaCuCfcAfuggugsgsa	2250	UCCACCAUGGAGUACCCUCUCAGC	2698
AD-1700819.1	cscsauggAfgUfAfCfcucucagcuL96	1803	asAfsgcdTg(Agn)gagguaCfuCfcauggsusg	2251	CACCAUGGAGUACCUUCUCAGCUC	2699
AD-1700821.1	asusggagUfaCfCfUfcucagcucuL96	1804	asAfsgadGc(Tgn)gagaggUfaCfuccausgsg	2252	CCAUGGAGUACCUCUCAGCUCUG	2700
AD-1700822.1	ususacucAfgGfUfCfaguaucuaauL96	1805	asUfsuadGa(Tgn)acugacCfuGfguaasgsu	2253	ACUUACUCAGGUCAUAUCUAAU	2701
AD-1700824.1	uscsaguaUfcUfAfAfuaaagcucuL96	1806	asGfsagdCu(Tgn)auauuaGfaUfacugascsc	2254	GGUCAGUAUCUAAUAUAAGCUCG	2702
AD-1700825.1	csasguauCfuAfAfUfauaagcucguL96	1807	asCfsgadGc(Tgn)uauauuAfgAfuaucgsasc	2255	GUCAGUAUCUAAUAUAAGCUCGG	2703
AD-1700826.1	asgsuaucUfaAfUfAfuaagcucgguL96	1808	asCfscgdAg(Cgn)uuauauUfaGfauacusgsa	2256	UCAGUAUCUAAUAUAAGCUCGGA	2704
AD-1700828.1	uscsuaauAfuAfAfGfcucggaguuuL96	1809	asAfsacdTc(Cgn)gagcuuAfuAfuagasusa	2257	UAUCUAAUAUAAGCUCGGAGUUU	2705
AD-1700829.1	csusaauaUfaAfGfCfucggaguuuuL96	1810	asAfsaadCu(Cgn)cgagcuUfaUfauuagsasu	2258	AUCUAAUAUAAGCUCGGAGUUUG	2706
AD-1700830.1	usasagcuCfGfAfGfuuggagcgguL96	1811	asCfscgdTc(Cgn)aaacucCfGfGcuuasusa	2259	UAUAAGCUCGGAGUUGGACGGA	2707
AD-1700831.1	asasgcucGfgAfGfUfuuggagcgguL96	1812	asUfscddGu(Cgn)caaacuCfcGfagcuusasu	2260	AUAAGCUCGGAGUUGGACGGA	2708
AD-1700832.1	gsgsaguuUfgGfAfCfaggaggucuuL96	1813	asAfsgadCc(Cgn)uccgucCfaAfacuccsgsa	2261	UCGGAGUUUGGACGAGGGGUCUG	2709
AD-1700833.1	usgsgacgGfaGfGfGfucuggaccuuL96	1814	asAfsggdTc(Cgn)agaccUfcCfguccasasa	2262	UUUGGACGGAGGGUUGGACCUC	2710
AD-1700834.1	asgscgacCfuUfUfCfcgugucuguL96	1815	asAfsacdGa(Cgn)acggaaAfgGfucgusgsg	2263	CCAGCGACCUUCCGUGUCUGUG	2711
AD-1700837.1	csasagcgGfaCfCfAfuccggaaaguL96	1816	asCfsuudTc(Cgn)ggauggUfcCfGcuugsusg	2264	CACAAGCGGACCAUCCGGAAAGG	2712
AD-1700838.1	csasuccgGfaAfAfGfgccugacaguL96	1817	asCfsugdTc(Agn)ggccuuUfcCfGgaugsgsu	2265	ACCAUCCGGAAAGGCCUGACAGC	2713
AD-1700839.1	asgsgagcUfgCfUfAfGCCaaagcauL96	1818	asUfsgcdTu(Tgn)ggcuagCfaGfcuccusgsg	2266	CCAGGAGCUGCUAGCCAAAGCAU	2714
AD-1700840.1	gsgsagcuGfcUfAfGfccaagcauuL96	1819	asAfsugdCu(Tgn)uggcuAGfcAfgcuusgsg	2267	CAGGAGCUGCUAGCCAAAGCAU	2715
AD-1700841.1	gsasgcugCfuAfGfCfcaaagcauuuL96	1820	asAfsaudGc(Tgn)uuggcuAfgCfagcuscscu	2268	AGGAGCUGCUAGCCAAAGCAUUG	2716
AD-1700842.1	asgscugcUfaGfCfCfaaagcauuguL96	1821	asCfsaadTg(Cgn)uuuggcUfaGfcagcuscsc	2269	GGAGCUGCUAGCCAAGCAUUGG	2717

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700843.1	csusagccAfaAfGfC fauuggagacuL96	1822	asGfsucdTc(Cgn)aaugcu UfuGfgcuagscsa	2270	UGCUAGCCAAAGCA UUGGAGACC	2718
AD-1700844.1	usasgccaAfaGfCfA fuuggagaccuL96	1823	asGfsgudCu(Cgn)caaugc UfuUfggcuasgsc	2271	GCUAGCCAAAGCAU UGGAGACCC	2719
AD-1700845.1	asgscauuGfgAfGfA fccuacugcuL96	1824	asGfscadGu(Agn)gggucu CfcAfaugcususu	2272	AAAGCAUUGGAGAC CCUACUGCU	2720
AD-1700846.1	gsascauuGfaGfAfC fccuacugcuL96	1825	asAfsgcdAg(Tgn)aggguc UfcCfaaugcsusu	2273	AAGCAUUGGAGACC CUACUGCUG	2721
AD-1700848.1	asusuggaGfaCfCfC fuacugcugauL96	1826	asUfscadGc(Agn)guaggg UfcUfccauasgsc	2274	GCAUUGGAGACCCU ACUGCUGAA	2722
AD-1700850.1	gsgsagacCfcUfAfC fugcugaauguL96	1827	asCfsaudTc(Agn)gcagua GfgGfucuccsasa	2275	UUGGAGACCCUACU GCUGAAUGG	2723
AD-1700851.1	gsasgaccCfuAfCfU fgcugaaugguL96	1828	asCfscadTu(Cgn)agcagu AfgGfgucucscsa	2276	UGGAGACCCUACUG CUGAAUGGA	2724
AD-1700852.1	ascscuaCfuGfCfU fgaaggaguuL96	1829	asAfscudCc(Agn)uucagc AfgUfaggguscsu	2277	AGACCCUACUGCUG AAUGGAGUG	2725
AD-1700853.1	cscscuacUfgCfUfG faauggaguguL96	1830	asCfsacdTc(Cgn)auucag CfaGfuagggsusc	2278	GACCCUACUGCUGA AUGGAGUGC	2726
AD-1700854.1	cscsuacuGfcUfGfA fauggagugcuL96	1831	asGfscadCu(Cgn)cauuc GfcAfguaggsgsu	2279	ACCCUACUGCUGAA UGGAGUGCU	2727
AD-1700855.1	csusacugCfuGfAfA fuggagugcuL96	1832	asAfsgcdAc(Tgn)ccauuc AfgCfaguagsgsg	2280	CCCUACUGCUGAAU GGAGUGCUA	2728
AD-1700856.1	usascugcUfgAfAfU fggagugcuauL96	1833	asUfsagdCa(Cgn)uccauu CfaGfcaguasgsg	2281	CCUACUGCUGAAUG GAGUGCUAA	2729
AD-1700858.1	gscsugaaUfgGfAfG fugcuaaccuL96	1834	asGfsggdTu(Agn)gcacuc CfaUfucagesasg	2282	CUGCUGAAUGGAGU GCUAACCCU	2730
AD-1700859.1	csusgaauGfgAfGfU fgcuaaccuL96	1835	asAfsggdGu(Tgn)agcacu CfcAfuucagscsa	2283	UGCUGAAUGGAGUG CUAACCUG	2731
AD-1700860.1	usgsaaugGfaGfUfG fcuaaccuL96	1836	asCfsagdGg(Tgn)uagcac UfcCfaucasgsc	2284	GCUGAAUGGAGUGC UAACCUGG	2732
AD-1700863.1	usgsagugGfcUfAf AfccugugcuL96	1837	asGfscadCc(Agn)ggguua GfcAfcuccasusu	2285	AAUGGAGUGCUAAC CCUGGUGCU	2733
AD-1700864.1	gsgsagugCfuAfAfC fccugugcuL96	1838	asAfsgcdAc(Cgn)aggguu AfgCfacuccsasu	2286	AUGGAGUGCUAACC CUGGUGCUA	2734
AD-1700865.1	gsasgugcUfaAfCfC fcuggugcuauL96	1839	asUfsagdCa(Cgn)cagggu UfaGfcacucscsa	2287	UGGAGUGCUAACCC UGGUGCUAG	2735
AD-1700867.1	asascuuGfgUfGfC fuaggagagguL96	1840	asCfscudCc(Tgn)cuagca CfcAfggguuasg	2288	CUAACCUGGUGCU AGAGGAGGA	2736
AD-1700868.1	cscscuggUfgCfUfA fgaggaggaauL96	1841	asAfsudCu(Cgn)cucuag CfaCfcagggsusu	2289	AACCUGGUGCUAG AGGAGGAUG	2737
AD-1700869.1	gsgsugcuAfgAfGf GfaggaggaauL96	1842	asUfsudCa(Tgn)ccuccu CfuAfgcaccsag	2290	CUGGUGCUAGAGGA GGAUGGAAC	2738
AD-1700870.1	gsusgcuGfaGfGfA fggauggaacuL96	1843	asGfsuudCc(Agn)uccucc UfcUfagcacscsa	2291	UGGUGCUAGAGGAG GAUGGAACU	2739
AD-1700871.1	csusagagGfaGfGfA fuggaacugcuL96	1844	asGfscadGu(Tgn)ccaucc UfcCfucuascsa	2292	UGCUAGAGGAGGAU GGAACUGCA	2740
AD-1700872.1	gsasggauGfgAfAfC fugcaguggauL96	1845	asUfscddAc(Tgn)gcagu CfcAfuucccsesu	2293	AGGAGGAUGGAACU GCAGUGGAC	2741

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700873.1	gsgsauggAfaCfUfGfcaguggacauL96	1846	asUfsgudCc(Agn)cugcagUfuCfcauccsusc	2294	GAGGAUGGAACUGCAGUGGACAG	2742
AD-1700874.1	gsasuggaAfcUfGfCfaguggacaguL96	1847	asCfsugdTc(Cgn)acugcaGfuUfccaucscsu	2295	AGGAUGGAACUGCAGUGGACAGU	2743
AD-1700875.1	asusggaaCfuGfCfAfguggacaguuL96	1848	asAfsudGu(Cgn)cacugcAfgUfuccaucscsc	2296	GGAUGGAACUGCAGUGGACAGUG	2744
AD-1700876.1	usgsgaacUfgCfAfGfuggacaguguL96	1849	asCfsacdTg(Tgn)ccacugCfaGfuuccasusc	2297	GAUGGAACUGCAGUGGACAGUGA	2745
AD-1700877.1	gsasacugCfaGfUfGfgacagugaguL96	1850	asCfsudAc(Tgn)guccacUfgCfaguucscsa	2298	UGGAACUGCAGUGGACAGUGAGG	2746
AD-1700878.1	ascugcaGfuGfGfAfcagugaggauL96	1851	asUfscddTc(Agn)cuguccAfcUfgcagususc	2299	GAACUGCAGUGGACAGUGAGGAC	2747
AD-1700879.1	csusgcagUfgGfAfCfagugaggacuL96	1852	asGfsudCu(Cgn)acugucCfaCfugcagsusu	2300	AACUGCAGUGGACAGUGAGGACU	2748
AD-1700880.1	usgscaguGfgAfCfAfgugaggacuuL96	1853	asAfsudCc(Tgn)cacuguCfcAfcugcasgsu	2301	ACUGCAGUGGACAGUGAGGACUU	2749
AD-1700881.1	gscsagugGfaCfAfGfugaggacuuuL96	1854	asAfsagdTc(Cgn)ucacugUfcCfacugcsasg	2302	CUGCAGUGGACAGUGAGGACUUC	2750
AD-1700882.1	csasguggAfcAfGfUfgaggacuucuL96	1855	asGfsaadGu(Cgn)cucacuGfuCfcacugscsa	2303	UGCAGUGGACAGUGAGGACUUCU	2751
AD-1700883.1	asgsuggaCfaGfUfGfaggacuucuuL96	1856	asAfsagdAg(Tgn)ccucacUfgUfccacusgsc	2304	GCAGUGGACAGUGAGGACUUCU	2752
AD-1700885.1	usgsgacaGfuGfAfGfgacuucuucuL96	1857	asGfsaadGa(Agn)guccucAfcUfguccasesu	2305	AGUGGACAGUGAGGACUUCUCC	2753
AD-1700887.1	csasgugaGfgAfCfUfucuuccagcuL96	1858	asGfscudGg(Agn)agaaguCfcUfccagususc	2306	GACAGUGAGGACUUCUCCAGCU	2754
AD-1700888.1	usgsaggaCfuUfCfUfuccagcugcuL96	1859	asGfscadGc(Tgn)ggaagaAfgUfccucasesu	2307	AGUGAGGACUUCUCCAGCUGCU	2755
AD-1700889.1	gsasggacUfuCfUfUfccagcugcuL96	1860	asAfsgcdAg(Cgn)uggaagAfaGfuccucsasc	2308	GUGAGGACUUCUCCAGCUGCUG	2756
AD-1700891.1	gsgsacuuCfuUfCfCfagcugcugguL96	1861	asCfscadGc(Agn)gcuggaAfgAfaguccsusc	2309	GAGGACUUCUCCAGCUGCUGGA	2757
AD-1700892.1	uscsuuccAfgCfUfGfcuggaggauuL96	1862	asAfsudCu(Cgn)cagcagCfuGfgaagasag	2310	CUUCUCCAGCUGCUGGAGGAUG	2758
AD-1700893.1	csasgcugCfuGfGfAfggaugacacuL96	1863	asGfsugdTc(Agn)uccuccAfgCfagcugsgsa	2311	UCCAGCUGCUGGAGGAUGACACG	2759
AD-1700894.1	gsesugcuGfgAfGfGfaugacacguuL96	1864	asAfsagdTg(Tgn)cauccuCfcAfgcagsusg	2312	CAGCUGCUGGAGGAUGACACGUG	2760
AD-1700895.1	csusggagGfaUfGfAfcagugccuuL96	1865	asAfsaggdCa(Cgn)gugucaUfcUfccagscsa	2313	UGCUGGAGGAUGACACGUGCCUG	2761
AD-1700897.1	gsgsaugaCfaCfGfUfgccugaugguL96	1866	asCfscadTc(Agn)ggcagUfgUfccaucsusc	2314	GAGGAUGACACGUGCCUGAUGGU	2762
AD-1700898.1	gsasugacAfcGfUfGfccugaugguuL96	1867	asAfsacdAu(Cgn)aggcacGfuGfucaucscsu	2315	AGGAUGACACGUGCCUGAUGGUG	2763
AD-1700899.1	gsascagUfgCfCfUfgaugguguuuL96	1868	asAfsacdAc(Cgn)aucaggCfaCfugugcsasu	2316	AUGACACGUGCCUGAUGGUGUUG	2764
AD-1700900.1	gsesagucUfgGfUfCfagagcuggauL96	1869	asUfscddAg(Cgn)ucugacCfaGfacugcsasa	2317	UUGCAGUCUGGUCAAGCUGGAG	2765

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700902.1	usascaagGfaGfUfGfgagugcuguuL96	1870	asAfsacdGc(Agn)cuccacUfcCfuuguasgsg	2318	CCUACAAGGAGUGGAGUGCUGUC	2766
AD-1700903.1	ascsaaggAfgUfGfGfagugcugucuL96	1871	asGfsacdAg(Cgn)acuccaCfuCfcuugusag	2319	CUACAAGGAGUGGAGUGCUGUCA	2767
AD-1700904.1	asasggagUfgGfAfGfugcugucauuL96	1872	asAfsugdAc(Agn)gcacucCfaCfuccuusgsu	2320	ACAAGGAGUGGAGUGCUGUCAUA	2768
AD-1700905.1	gsgsagugGfaGfUfGfcugucuuL96	1873	asAfsuadTg(Agn)cagcacUfcCfacuccsusu	2321	AAGGAGUGGAGUGCUGUCAUAUG	2769
AD-1700906.1	usgsagugGfcUfGfUfcauauaggccuL96	1874	asGfsgcdCa(Tgn)augacaGfcAfcuccasesu	2322	AGUGGAGUGCUGUCUAUUGGCCU	2770
AD-1700907.1	gsgsagugCfuGfUfCfauauaggccuL96	1875	asAfsaggdCc(Agn)uauagacAfgCfacuccsasc	2323	GUGGAGUGCUGUCAUAUGGCCUG	2771
AD-1700908.1	gsasugugUfgUfCfAfuaugggcuguL96	1876	asCfsagdGc(Cgn)auaugaCfaGfcacucscsa	2324	UGGAGUGCUGUCAUAUGGCCUGG	2772
AD-1700909.1	asgsugcuGfuCfAfUfauggccugguL96	1877	asCfscadGg(Cgn)cauauGfAfgcacuscsc	2325	GGAGUGCUGUCAUAUGGCCUGGG	2773
AD-1700912.1	gscsugucAfuAfUfGfgccugggacuL96	1878	asGfsucdCc(Agn)ggccauAfuGfcagcsasc	2326	GUGCUGUCAUAUGGCCUGGGACG	2774
AD-1700913.1	csusgucaUfaUfGfGfccugggacguL96	1879	asCfsgudCc(Cgn)aggccaUfaUfgacagsca	2327	UGCUGUCAUAUGGCCUGGGACGG	2775
AD-1700915.1	csasagcaCfaGfCfAfaggacaucguL96	1880	asCfsgadTg(Tgn)ccuugcUfgUfgcuugsgsg	2328	CCCAAGCACAGCAAGGACAUCGC	2776
AD-1700916.1	asusucacCfuUfUfGfacguguacauL96	1881	asUfsgudAc(Agn)cguaaAfgGfguaauscsg	2329	CGAUUACCUUUGACGUGUACAA	2777
AD-1700917.1	ususcaccUfuUfGfAfeguguacaauL96	1882	asUfsugdTa(Cgn)acgucaAfaGfgugaasusc	2330	GAUUCACCUUUGACGUGUACAAG	2778
AD-1700918.1	uscsaccUfuGfAfCfuguguacaaguL96	1883	asCfsuudGu(Agn)cagcucAfaAfggugasasu	2331	AUUCACCUUUGACGUGUACAAGC	2779
AD-1700919.1	csascuuUfgAfCfGfuguacaagcuL96	1884	asGfscudTg(Tgn)acagcuCfaAfggugsasa	2332	UUCACCUUUGACGUGUACAAGCA	2780
AD-1700920.1	cscsuuugAfcGfUfGfuacaagcaauL96	1885	asUfsugdCu(Tgn)guacacGfuCfaaaggsusg	2333	CACCUUUGACGUGUACAAGCAAA	2781
AD-1700922.1	usgsagcuGfuAfCfAfagcaaaaccuL96	1886	asGfsgudTu(Tgn)gcuuguAfcAfcgucasasa	2334	UUUGACGUGUACAAGCAAAACCC	2782
AD-1700923.1	gsascgugUfaCfAfAfgcaaaaccuL96	1887	asGfsggdTu(Tgn)ugcuugUfaCfacgucsasa	2335	UUGACGUGUACAAGCAAAACCCU	2783
AD-1700924.1	ascsguguAfcAfAfGfcaaaaccuL96	1888	asAfsaggdGu(Tgn)uugcuuGfuAfcaguscasa	2336	UGACGUGUACAAGCAAAACCCUC	2784
AD-1700925.1	csgsuguaCfaAfGfCfaaaaccucuL96	1889	asGfsagdGg(Tgn)uuugcuUfgUfacacgsusc	2337	GACGUGUACAAGCAAAACCCUCG	2785
AD-1700929.1	csasagcaAfaAfCfCfcucgagaccuL96	1890	asGfsgudCu(Cgn)gagggUfuUfgcuugsusa	2338	UACAAGCAAAACCCUCGAGACCU	2786
AD-1700930.1	asasgcaaAfaCfCfCfucgagaccuL96	1891	asAfsaggdTc(Tgn)cgaggUfuUfugcuusgsu	2339	ACAAGCAAAACCCUCGAGACCUC	2787
AD-1700931.1	asgscaaaAfcCfCfUfcgagaccucuL96	1892	asGfsagdGu(Cgn)ucgaggGfuUfuugcususg	2340	CAAGCAAAACCCUCGAGACCUCU	2788
AD-1700934.1	gsasgaccUfcUfUfUfggcagccuguL96	1893	asCfsagdGc(Tgn)gccaaaGfaGfgucucgsa	2341	UCGAGACCUCUUUGGCAGCCUGA	2789

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700936.1	cscsucuuUfgGfCfAfgccugaauL96	1894	asCfsaudTc(Agn)ggcugcCfaAfaagagsusc	2342	GACCUCUUUGGCAGCCUGAAUGU	2790
AD-1700937.1	csuscuuuGfgCfAfGfccugaauuuL96	1895	asAfscaadTu(Cgn)aggcugCfcAfaagagsgsu	2343	ACCUCUUUGGCAGCCUGAAUGUC	2791
AD-1700938.1	csusuuggCfaGfCfCfugaauugauL96	1896	asUfsgadCa(Tgn)ucaggcUfgCfcaagsasg	2344	CUCUUUGGCAGCCUGAAUGUCA	2792
AD-1700939.1	ususuggcAfgCfCfUfgaauucauL96	1897	asUfsugdAc(Agn)uucaggCfuGfcaasgsa	2345	UCUUUGGCAGCCUGAAUGUCAA	2793
AD-1700940.1	usgsgcagCfcUfGfAfaugcaauL96	1898	asCfsuudTg(Agn)cauucagGfGfugccasasa	2346	UUUGGCAGCCUGAAUGUCAAGC	2794
AD-1700942.1	gscsagccUfgAfAfUfgucaagccuL96	1899	asGfsgcdTu(Tgn)gacauuUfgGfugccscsa	2347	UGGCAGCCUGAAUGUCAAGCCA	2795
AD-1700943.1	csasgccUfgAfAfUfgucaagccuL96	1900	asUfsggdCu(Tgn)ugacauUfcAfggcugscsc	2348	GGCAGCCUGAAUGUCAAGCCAC	2796
AD-1700944.1	asgsgcugAfaUfGfUfcaagccacuL96	1901	asGfsugdGc(Tgn)uugacaUfuCfagggcugsc	2349	GCAGCCUGAAUGUCAAGCCACA	2797
AD-1700947.1	asusgucaAfaGfCfCfacauucacuL96	1902	asGfsuadGa(Agn)uguggcUfuUfgacaususc	2350	GAAUGUCAAGCCAUAUUCUACG	2798
AD-1700948.1	asgscacAfUfCfUfacgggcucuL96	1903	asAfsgadGc(Cgn)cguaaAfUfgggcucusu	2351	AAAGCCACAUUCUACGGGCUCUA	2799
AD-1700949.1	gscscacaUfuCfUfAfcgggcucuL96	1904	asUfsagdAg(Cgn)ccguagAfaUfgggcucusu	2352	AAGCCACAUUCUACGGGCUCUAC	2800
AD-1700950.1	csascauuCfuAfCfGfggcucuacuL96	1905	asAfsagdAg(Agn)gcccguAfgAfaugugsgsc	2353	GCCACAUUCUACGGGCUCUACUC	2801
AD-1700952.1	csasuucuAfcGfGfGfcucuacuL96	1906	asAfsagdGu(Agn)gagcccGfuAfgaugsusc	2354	CACAUUCUACGGGCUCUACUCUA	2802
AD-1700953.1	asusucuaCfGfGfCfucacuacuL96	1907	asUfsagdAg(Tgn)agagccCfGfUfagaugsu	2355	ACAUUCUACGGGCUCUACUCUAU	2803
AD-1700955.1	uscsuacGfgCfUfCfuacuauL96	1908	asCfsaudAg(Agn)guagagCfcCfugaugasu	2356	AUUCUACGGGCUCUACUCUAUGA	2804
AD-1700957.1	ascsgggcUfcUfAfCfucuaugauL96	1909	asAfsadCa(Tgn)agaguaGfaGfcccusag	2357	CUACGGGCUCUACUCUAUGAGUU	2805
AD-1700958.1	gsgsgcucUfaCfUfCfuaugaguL96	1910	asCfsaadCu(Cgn)auagagUfaGfagccsgsu	2358	ACGGGCUCUACUCUAUGAGUUGU	2806
AD-1700959.1	gsgscucuAfcUfCfUfaugaguL96	1911	asAfscaadAc(Tgn)cauagaGfuAfgagccscg	2359	CGGGCUCUACUCUAUGAGUUGUG	2807
AD-1700960.1	csusacucUfaUfGfAfguugugacuL96	1912	asAfsagdCa(Cgn)aacucaUfaGfaguagsag	2360	CUCUACUCUAUGAGUUGUGACUU	2808
AD-1700961.1	usascucuAfuGfAfGfuugugacuuL96	1913	asAfsagdTc(Agn)caacucAfuAfgaguasgsa	2361	UCUACUCUAUGAGUUGUGACUUU	2809
AD-1700962.1	ascsucuaUfgAfGfUfugugacuuL96	1914	asAfsaadGu(Cgn)acaacuCfaUfagagusag	2362	CUACUCUAUGAGUUGUGACUUUC	2810
AD-1700963.1	csuscuauGfaGfUfUfgugacuuL96	1915	asGfsaadAg(Tgn)cacaacUfcAfuagagsusa	2363	UACUCUAUGAGUUGUGACUUUCA	2811
AD-1700965.1	csusaugaGfuUfGfUfgacuucauL96	1916	asUfsugdAa(Agn)gucacaAfcUfcuagagsag	2364	CUCUAUGAGUUGUGACUUUCAAG	2812
AD-1700967.1	gsasguugUfgAfCfUfuucaaggacuL96	1917	asGfsucdCu(Tgn)gaaaguCfaCfaacucsasu	2365	AUGAGUUGUGACUUUCAAGGACU	2813

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1700968.1	asgsuuguGfaCfUfUfucaaggacuuL96	1918	asAfsugdCc(Tgn)ugaaagUfcAfcacuscasa	2366	UGAGUUGUGACUUU CAAGGACUU	2814
AD-1700969.1	ususggccCfaAfAfGfaaaguacucuL96	1919	asGfsagdTa(Cgn)uuucuuUfgGfgccaasgsu	2367	ACUUGGCCCAAAGA AAGUACUCA	2815
AD-1700970.1	usgsgcccAfaAfGfAfaaguacucuuL96	1920	asUfsgadGu(Agn)cuuucuUfuGfggccasasg	2368	CUUGGCCCAAAGAA AGUACUCAG	2816
AD-1700971.1	gsgscccaAfaGfAfAfaguacucaguL96	1921	asCfsugdAg(Tgn)acuuucUfuUfgggccsasa	2369	UUGGCCCAAAGAAA GUACUCAGG	2817
AD-1700973.1	cscscaaaGfaAfAfGfuacucaggguL96	1922	asCfscddTg(Agn)guacuuUfcUfuugggscsc	2370	GGCCCAAAGAAAGU ACUCAGGGA	2818
AD-1700975.1	asasagaaAfgUfAfCfucaggaggcuL96	1923	asGfscudCc(Cgn)ugaguaCfuUfucuuusgsg	2371	CCAAAGAAAGUACU CAGGGAGCU	2819
AD-1700976.1	asasgaaaGfuAfCfUfcaggaggcuL96	1924	asAfsgcdTc(Cgn)cugaguAfcUfuucuuusg	2372	CAAAGAAAGUACUC AGGGAGCUC	2820
AD-1700977.1	asgsaaagUfaCfUfCfaggaggcuL96	1925	asGfsagdCu(Cgn)ccugagUfaCfuucucusu	2373	AAAGAAAGUACUCA GGGAGCUCC	2821
AD-1700978.1	asgsuacuCfaGfGfGfagcuccuucuL96	1926	asGfsaadGg(Agn)gcuccUfgAfguacususu	2374	AAAGUACUCAGGGA GCUCCUUCG	2822
AD-1700980.1	asgsggagCfuCfCfUfucguuggacuL96	1927	asGfsucdCa(Agn)cgaaggAfgCfuccusgsa	2375	UCAGGGAGCUCCU CGUUGGACC	2823
AD-1700981.1	gsgsagcuCfcUfUfCfguuggaccuuL96	1928	asAfsggdTc(Cgn)aacgaaGfgAfgcuccscsu	2376	AGGGAGCUCCUUCG UUGGACCUC	2824
AD-1700982.1	ususcguuGfgAfCfCfuccacacuguL96	1929	asCfsagdTg(Tgn)ggagguCfcAfacgaasgsg	2377	CCUUCGUUGGACCU CCACACUGC	2825
AD-1700984.1	csgsuuggAfcCfUfCfcaacucguL96	1930	asAfsgcdAg(Tgn)guggagGfuCfcaacgsasa	2378	UUCGUUGGACCUC ACACUGCUG	2826
AD-1700985.1	gsascucCfaCfAfCfugcugcaaguL96	1931	asCfsuudGc(Agn)gcagugUfgGfaggucscsa	2379	UGGACCUCACACU GCUGCAAGG	2827
AD-1700986.1	gsgscugGfgCfCfAfuauugcuL96	1932	asAfsgcdAa(Cgn)auaugGfcCfaggccsusu	2380	AAGGCCUGGGCCAU AUGUUGCUG	2828
AD-1700988.1	gsgsgccaUfaUfGfUfugcugggaauL96	1933	asUfsucdCc(Agn)gcaacaUfaUfgggccsag	2381	CUGGGCCAU AUGU GCUGGGAU	2829
AD-1700989.1	gsgsccauAfuGfUfUfgcugggaauL96	1934	asAfsuudCc(Cgn)agcaacAfuAfuaggccscsa	2382	UGGGCCAU AUGU CUGGGAU	2830
AD-1700990.1	cscsauauGfuUfGfCfugggaauuuL96	1935	asAfsaadTu(Cgn)ccagcaAfcAfuauaggscsc	2383	GGCCAU AUGUUGCU GGGAUUUC	2831
AD-1700991.1	csasuauGfuGfCfUfgggaauuuL96	1936	asGfsaadAu(Tgn)cccagcAfaCfauaugsgsc	2384	GCCAU AUGUUGCUG GGAAUUUC	2832
AD-1700992.1	usgsuugcUfgGfGfAfauuuccuccuL96	1937	asGfsagdGg(Agn)aaauccCfaGfcaacasusa	2385	UAUGUUGCUGGGAA UUUCCUCA	2833
AD-1700995.1	usgsggaaUfuUfCfCfuccacccuuL96	1938	asAfsagdGg(Tgn)ggaggaAfaUfuccasgsc	2386	GCUGGGAAUUUCCU CCACCCUUC	2834
AD-1700997.1	ususcucCfaCfCfCfuucgucauguL96	1939	asCfsaudGa(Cgn)gaagggUfgGfaggaasasu	2387	AUUUCCUCCACCCU UCGUCAUGC	2835
AD-1700998.1	asasgggcCfGfCfCfUfccauuccuauL96	1940	asUfsagdGa(Agn)uggaggCfGfcccuscsu	2388	AGAAGGGCCGCCUC CAUCCUAC	2836
AD-1701001.1	gscscgccUfcCfAfUfuuccuacuaauL96	1941	asUfsuadGu(Agn)ggaugGfaGfgggcscsc	2389	GGGCCGCCUCCA CCUACUAAG	2837

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1701002.1	cscsgccuCfcAfUfUfccuacuaaguL96	1942	asCfsuudAg(Tgn)aggaauGfgAfgggcgscsc	2390	GGCCGCCUCCAUUC CUACUAAGG	2838
AD-1701003.1	cscscacuGfcAfAfAfgacuaugacuL96	1943	asGfsucdAu(Agn)gucuuuGfcAfgugggsusc	2391	GACCCACUGCAAAG ACUAUGACA	2839
AD-1701004.1	cscsacugCfaAfAfGfacuauagacuL96	1944	asUfsgudCa(Tgn)agucuuUfgCfaguggsgsu	2392	ACCCACUGCAAAGA CUAUGACAG	2840
AD-1701005.1	csascugcAfaAfGfAfcuauagacaguL96	1945	asCfsugdTc(Agn)uagucuUfuGfcagugsgsg	2393	CCCACUGCAAAGAC UAUGACAGC	2841
AD-1701007.1	asasgacuAfuGfAfCfagcaucaaaL96	1946	asUfsuudGa(Tgn)gcugucAfuAfgucuuusug	2394	CAAAGACUAUGACA GCAUCAAAU	2842
AD-1701009.1	gsascagcAfuCfAfAfauuucaggauL96	1947	asUfscddTg(Agn)aaauugAfuGfcugucsasu	2395	AUGACAGCAUCAAA UUUCAGGAC	2843
AD-1701011.1	csasgcauCfaAfAfUfuucaggaccuL96	1948	asGfsgudCc(Tgn)gaaauUfgAfugcugsusc	2396	GACAGCAUCAAAUU UCAGGACCU	2844
AD-1701012.1	asgscaucAfaAfUfUfucaggaccuuL96	1949	asAfsugdTc(Cgn)ugaaauUfuGfaugcugsu	2397	ACAGCAUCAAAUUU CAGGACCUG	2845
AD-1701013.1	gsascauAfaUfUfUfcaggaccuguL96	1950	asCfsagdGu(Cgn)cugaaaUfuUfgaugcsusg	2398	CAGCAUCAAAUUUC AGGACCUGC	2846
AD-1701014.1	csasucaaAfuUfUfCfaggaccugcuL96	1951	asGfscadGg(Tgn)ccugaaAfuUfugaugscsu	2399	AGCAUCAAAUUUCA GGACCUGCA	2847
AD-1701016.1	asusuucaGfgAfCfCfugcagacaguL96	1952	asCfsugdTc(Tgn)gcagguCfcUfgaaususu	2400	AAAUUUCAGGACCU GCAGACAGU	2848
AD-1701017.1	ususucagGfaCfCfUfgcagacaguL96	1953	asAfsudGu(Cgn)ugcaggUfcCfugaaususu	2401	AAUUUCAGGACCUG CAGACAGUA	2849
AD-1701019.1	csasggacCfuGfCfAfgacaguacauL96	1954	asUfsgudAc(Tgn)gucugcAfgGfuccugsasa	2402	UUCAGGACCUGCAG ACAGUACAG	2850
AD-1701020.1	gsascugCfaGfAfCfaguacaggcuL96	1955	asGfscddTg(Tgn)acugucUfgCfaggucscsu	2403	AGGACCUGCAGACA GUACAGGCU	2851
AD-1701022.1	cscsugcaGfaCfAfGfuacaggcuauL96	1956	asUfsagdCc(Tgn)guacugUfuUfgcaggsusc	2404	GACCUGCAGACAGU ACAGGCUAG	2852
AD-1701023.1	csusgcagAfcAfGfUfacaggcuaguL96	1957	asCfsuadGc(Cgn)uguacuGfuCfugcagsgsu	2405	ACCUGCAGACAGUA CAGGCUAGA	2853
AD-1701024.1	usgscagaCfaGfUfAfcaggcuagauL96	1958	asUfscudAg(Cgn)cuguacUfuUfcugcasgsg	2406	CCUGCAGACAGUAC AGGCUAGAU	2854
AD-1701026.1	csasgacaGfuAfCfAfggcuagauauL96	1959	asUfsaudCu(Agn)gccuguAfcUfgucugscsa	2407	UGCAGACAGUACAG GCUAGAUAA	2855
AD-1701027.1	gsascaguAfcAfGfGfcuagauaacuL96	1960	asGfsuudAu(Cgn)uagccuGfuAfcugucsusg	2408	CAGACAGUACAGGC UAGAUAAACC	2856
AD-1701029.1	cscsuagcCfaCfCfUfuucuggccuuL96	1961	asAfsugdCc(Agn)gaaaggUfgGfcuaggsasa	2409	UUCCUAGCCACCUU UCUGGCCUC	2857
AD-1701031.1	csusuucuGfgCfCfUfccacuugcuL96	1962	asGfscadAg(Tgn)gggaggCfcAfgaaagsgsu	2410	ACCUUUCUGGCCUC CCACUUGCC	2858
AD-1701032.1	uscsgugcCfuCfCfCfacuugcccuL96	1963	asAfsugdGc(Agn)agugggAfgGfccagasasa	2411	UUUCUGGCCUCCCA CUUGCCCUG	2859
AD-1701033.1	gsgscaucUfuGfAfUfccagccuguL96	1964	asCfsagdGc(Tgn)gggaucAfaGfaugccsusg	2412	CAGGCAUCUUGAUC CCAGCCUGA	2860
AD-1701034.1	gsascauUfgAfUfCfccagccugauL96	1965	asUfscadGg(Cgn)ugggauCfaAfgaugcsesu	2413	AGGCAUCUUGAUCC CAGCCUGAC	2861

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1701035.1	ascscaaUGfcCfUfCfcuguccuauL96	1966	asUfsagdGg(Agn)caggagGfcAfuuggusag	2414	CUACCA AUGCCUCCUGUCCCUAA	2862
AD-1701037.1	asusgccuCfcUfGfUfccuaaacuuL96	1967	asAfsagdTu(Agn)gggacaGfgAfggcausug	2415	CAAUGCCUCCUGUCCCUAAACUC	2863
AD-1701038.1	gsescuccUfgUfCfCfcuaaacuccuL96	1968	asGfsgadGu(Tgn)uagggaCfaGfgaggcsasu	2416	AUGCCUCCUGUCCCUAAACUCCC	2864
AD-1720280.1	asasaaccuCfGfAfgaccucuuuuL96	1969	asdAsadAg(Agn)ggucudCgAfgGfguuuusgsc	2417	GCAAAACCCUCGAGACCUCUUUG	2865
AD-1720281.1	csusgggugAfGfAfucccagguuuL96	1970	asdAsadCc(Tgn)gggaduCuCfaGfccagsgsa	2418	AGGAAAGAGCAGAUCCCAGGUUU	2866
AD-1720282.1	gsusggacagUfGfAfggacuucuuL96	1971	asdAsadGa(Agn)guccudCaCfuGfuccacsug	2419	CAGUGGACAGUGAGGACUUCUUC	2867
AD-1720283.1	csasaaaccUfCfGfagaccucuuL96	1972	asdAsagadGg(Tgn)cucdGaGfgGfuuuugscsu	2420	AGCAAAACCCUCGAGACCUCUUU	2868
AD-1720284.1	csusgaaguCfAFAfagccacuuL96	1973	asdAsadTg(Tgn)ggcuudTgAfcAfuucagsgsc	2421	GCCUGAAUGUCAAA GCCACAUC	2869
AD-1720285.1	gsgscucuAfcUfCfUfaugaguuguL96	1974	asdAscAdAc(Tgn)cauadGaGfuAfgagccscsg	2422	CGGGCUCUACUCUAUGAGUUGUG	2870
AD-1720286.1	asasuggaguGfCfUfaaccucguuL96	1975	asdAscc(Agn)gdGguuadGcAfcUfccauuscsa	2423	UGAAUGGAGUGCUAACCCUGGUG	2871
AD-1720287.1	asasuggaguGfCfUfaaccuugguL96	1976	asdAsccAdAadGguuadGcAfcuccauuscsa	2424	UGAAUGGAGUGCUAACCCUGGUG	2872
AD-1720288.1	asasuggaguGfCfUfaacucugguL96	1977	asdAsccAdAgdAguuadGcAfcuccauuscsa	2425	UGAAUGGAGUGCUAACCCUGGUG	2873
AD-1720289.1	ascscuacCfuGfCfUfgaauaggauL96	1978	asdAscudCc(Agn)uucadGcAfgUfaggguscsu	2426	AGACCCUACUGCUGAAUGGAGUG	2874
AD-1720290.1	ascscugcagAfCfAfguacagguL96	1979	asdAsgdCc(Tgn)guacudGuCfuGfcagguscsu	2427	GGACCUGCAGACAGUACAGGCUA	2875
AD-1720291.1	csusgggaauUfUfCfcuccaccuuL96	1980	asdAsgdGg(Tgn)ggaggdAaAfuUfccagscsa	2428	UGCUGGGAAUUUCCUCCACCUU	2876
AD-1720292.1	gsgsgccgccUfCfCfaauccuacuuL96	1981	asdAsguadGg(Agn)augdGaGfgCfggcccsusu	2429	AAGGGCCGCCUCCA UCCUACUA	2877
AD-1720293.1	ususcuaaggGfCfUfcuacucuaauL96	1982	asdAsudAg(Agn)guagadGcCfcGfuagaasug	2430	CAUUCUACGGGCUCUACUCUAUG	2878
AD-1720294.1	gsesagacagUfAfcfaggcuagauL96	1983	asdAsudCu(Agn)gccugdTaCfuGfucugcsag	2431	CUGCAGACAGUACAGGCUAGUA	2879
AD-1720295.1	usgsgagaccCfUfAfcugcugaaauL96	1984	asdAsudTc(Agn)gcagudAgGfgUfccasasu	2432	AUUGGAGACCCUACUGCUGAAUG	2880
AD-1720296.1	ascscucuuUGfGfCfagccugaaauL96	1985	asdAsudTc(Agn)ggcugdCcAfaAfgagguscsu	2433	AGACCUCUUUGGCA GCCUGAAUG	2881
AD-1720297.1	csesuucugGfCfCfucccacuuguL96	1986	asdCsadAg(Tgn)gggagdGcCfaGfaaaggsug	2434	CACCUUUCUGGCCUCCCACUUGC	2882
AD-1720298.1	usgsgaacUfgCfAfgfuggacaguguL96	1987	asdCsadTg(Tgn)ccacdTgCfaGfuuccasusc	2435	GAUGGAACUGCAGUGGACAGUGA	2883
AD-1720299.1	asgsgaugacAfCfGfugccugauguL96	1988	asdCsadTc(Agn)ggcagdGuGfuCfauccuscsc	2436	GGAGGAUGACACGUGCCUGAUGG	2884
AD-1720300.1	gsasauggagUfGfCfuaaccugguL96	1989	asdCsagadGg(Tgn)uagdCaCfuCfcauucsasg	2437	CUGAAUGGAGUGCUAACCCUGGU	2885

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1720301.1	gsusgcugucAfUfAfuggccugggL96	1990	asdCscC(Agn)gdGccaudAuGfaCfagcacsusc	2438	GAGUGCUGUCAUAU GGCCUGGGA	2886
AD-1720302.1	gsusgcugucAfUfAfuggcuuggguL96	1991	asdCscdAadGccaudAuGfacagcacsusc	2439	GAGUGCUGUCAUAU GGCCUGGGA	2887
AD-1720303.1	gsusgcugucAfUfAfuggucugggL96	1992	asdCscdAgdAccaudAuGfacagcacsusc	2440	GAGUGCUGUCAUAU GGCCUGGGA	2888
AD-1720304.1	gscscCaaagAfAfAfguacucagguL96	1993	asdCscdTg(Agn)guacudTuCfuUfugggcsCsa	2441	UGGCCCAAAGAAAG UACUCAGGG	2889
AD-1720305.1	gsusacucagGfGfAfgcuccuucguL96	1994	asdCsgaadGg(Agn)gcudCcCfuGfaguacsusu	2442	AAGUACUCAGGGAG CUCCUUCGU	2890
AD-1720306.1	gsgsaauuucCfUfCfcaccuucguL96	1995	asdCsga(Agn)gdGguggdAgGfaAfauuccscsa	2443	UGGGAAUUUCCUCC ACCCUUCGU	2891
AD-1720307.1	gsgsaauuucCfUfCfcaccuucguL96	1996	asdCsgadAadGguggdAgGfaauuccscsa	2444	UGGGAAUUUCCUCC ACCCUUCGU	2892
AD-1720308.1	gsgsaauuucCfUfCfcacucucguL96	1997	asdCsgadAgdAguggdAgGfaauuccscsa	2445	UGGGAAUUUCCUCC ACCCUUCGU	2893
AD-1720309.1	gsusguacaaGfCfAfaaaccucguL96	1998	asdCsgagdGg(Tgn)uuudGcUfuGfuacacsgsu	2446	ACGUGUACAAGCAA AACCUCGA	2894
AD-1720310.1	csasgucuggUfCfAfgagcuggaguL96	1999	asdCsudCc(Agn)gcucudGaCfCfagacugscsa	2447	UGCAGUCUGGUCAG AGCUGGAGC	2895
AD-1720311.1	usgscuaaccCfUfGfgugcuagaguL96	2000	asdCsudCu(Agn)gcaccdAgGfgUfuagcascsu	2448	AGUGCUAACCCUGG UGCUAGAGG	2896
AD-1720312.1	uscsaaauuuCfAfGfgaccucgaguL96	2001	asdCsudGc(Agn)gguccdTgAfaAfuugasusg	2449	CAUCAAAUUUCAGG ACCUGCAGA	2897
AD-1720313.1	asgsaaccacCfCfAfgcccagaaguL96	2002	asdCsudTc(Tgn)gggcudGgGfuGfguucscsu	2450	AGAGAACCACCCAG CCCAGAAGU	2898
AD-1720314.1	csasaacuaaCfAfAfaaacaauuucuL96	2003	asdGsadAa(Tgn)guuuudTgUfuAfguuugsasg	2451	CUCAAAACUAACAAA ACAUUUCC	2899
AD-1720315.1	csasuggaguAfCfCfucucagcucuL96	2004	asdGsadGc(Tgn)gagagdGuAfcUfccaugsgsu	2452	ACCAUGGAGUACCU CUCAGCUCU	2900
AD-1720316.1	uscsguuggaCfCfUfccacacugcuL96	2005	asdGscdAg(Tgn)guggadGgUfcCfaacgasasg	2453	CUUCGUUGGACCUC CACACUGCU	2901
AD-1720317.1	ascscauggaGfUfAfccucucagcuL96	2006	asdGscdTg(Agn)gaggudAcUfcCfauggusgsg	2454	CCACCAUGGAGUAC CUCUCAGCU	2902
AD-1720368.1	gsgscagccuGfAfAfgucaaaagcuL96	2007	asdGscdTg(Tgn)gacaudTcAfgGfcugccsasa	2455	UUGGCAGCCUGAAU GUCAAAGCC	2903
AD-1720369.1	uscsuagccAfCfCfuucugggcuL96	2008	asdGsgdCc(Agn)gaaagdGuGfgCfuaggasasa	2456	UUUCCUAGCCACCU UUCUGGCCU	2904
AD-1720370.1	usgscugaauGfGfAfgugcuaaaccuL96	2009	asdGsgdTg(Agn)gcacudCcAfuUfcagcascsu	2457	ACUGCUGAAUGGAG UGCUAACCC	2905
AD-1720371.1	uscsaggaccUfGfCfagacaguacuL96	2010	asdGsudAc(Tgn)gucugdCaGfgUfccugasasa	2458	UUUCAGGACCUGCA GACAGUACA	2906
AD-1720372.1	ascsgaCaucAfAfAfuucaggacuL96	2011	asdGsudCc(Tgn)gaaudTuGfaUfgcuguscscsa	2459	UGACAGCAUCAAAU UUCAGGACC	2907
AD-1720373.1	ususcugggAfAfUfuuccuccacuL96	2012	asdGsudGg(Agn)ggaaadTuCfcCfagcaascsa	2460	UGUUGCUGGGAAUU UCCUCCACC	2908
AD-1720374.1	asasugccucCfUfGfuuccuaaacuL96	2013	asdGsudTu(Agn)gggacdAgGfaGfgcauusgsg	2461	CCAAUGCCUCCUGU CCCUAAACU	2909

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1720375.1	usugacgugUfAfCf aagcaaaacuL96	2014	asdGsudTu(Tgn)gcuugdTaCfaCfgucaasasg	2462	CUUUGACGUGUACA AGCAAAACC	2910
AD-1720376.1	gsgscgccuCfCfAf uuccuacuauL96	2015	asdTsadGu(Agn)ggaudGgAfgGfcggccscsu	2463	AGGGCCGCCUCCAU UCCUACUAA	2911
AD-1720377.1	ascsucagguCfAfGf uaucauuauL96	2016	asdTsadTu(Agn)gauacdTgAfcCfugagusasa	2464	UUACUCAGGUCAGU AUCUAAUAAU	2912
AD-1720378.1	usgsggccauAfUfGf uugcugggauL96	2017	asdTscdCc(Agn)gcaacdA uAfUfGfcccascsg	2465	CCUGGGCCAUAUGU UGCUGGGAA	2913
AD-1720379.1	usgscugucaUfAfUf ggcucgggauL96	2018	asdTscdCc(Agn)ggccadT aUfgAfcagcascsu	2466	AGUGCUGUCAUAUG GCCUGGGAC	2914
AD-1720380.1	usgsaguuguGfAfCf uuucaaggauL96	2019	asdTscdCu(Tgn)gaaagdT cAfcAfacucasusa	2467	UAUGAGUUGUGACU UUCAAGGAC	2915
AD-1720381.1	asgsgcccaaGfCfAfc agcaaggauL96	2020	asdTscdCu(Tgn)gcugudG cUfuGfggccscsu	2468	AGAGGCCCAAGCAC AGCAAGGAC	2916
AD-1720382.1	usgsuacaagCfAfAf aaccucggaL96	2021	asdTscg(Agn)gdGguuud TgCfuUfguacascsg	2469	CGUGUACAAGCAAA ACCCUCGAG	2917
AD-1720383.1	usgsuacaagCfAfAf aaccuucggaL96	2022	asdTscgdAadGguuudTgC fuuguacascsg	2470	CGUGUACAAGCAAA ACCCUCGAG	2918
AD-1720384.1	usgsuacaagCfAfAf aacucucggaL96	2023	asdTscgdAadGguuudTg Cfuuguacascsg	2471	CGUGUACAAGCAAA ACCCUCGAG	2919
AD-1720385.1	uscsuagagUfUfGf ugacuuuauL96	2024	asdTsgdAa(Agn)gucacdA aCfuCfauagagsu	2472	ACUCUAUGAGUUGU GACUUUCAA	2920
AD-1720386.1	asasagacuaUfGfAf cagcaucaauL96	2025	asdTsudGa(Tgn)gcugudC aUfaGfucuuusgsc	2473	GCAAAGACUAUGAC AGCAUCAA	2921

Example 2. *In vitro* screening of CIDEB siRNA

Experimental Methods

Cell culture and transfections:

5 Hepalcl7 cells are grown to near confluence at 37°C in an atmosphere of 5% CO₂ in Minimum Essential Medium Alpha (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Transfection is carried out by adding 14.8 µl of Opti-MEM plus 0.2 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5 µl of each siRNA duplex to an individual well in a 96-well plate. The mixture is then incubated at room temperature for 15 minutes. Eighty µl of

10 complete growth media without antibiotic containing ~2 x 10⁴ cells are then added to the siRNA mixture. Cells are incubated for 24 hours prior to RNA purification. Single dose experiments are performed at 10 nM, 1 nM and/or 0.1 nM final duplex concentration.

Panc-1 cells are grown to near confluence at 37°C in an atmosphere of 5% CO₂ in Minimum Essential Medium Alpha (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by

15 trypsinization. Transfection is carried out by adding 14.6 µl of Opti-MEM plus 0.4 µl of Lipofectamine 2000 per well to 5 µl of each siRNA duplex to an individual well in a 96-well plate. The mixture is then incubated

at room temperature for 15 minutes. Eighty μ l of complete growth media without antibiotic containing $\sim 1.5 \times 10^4$ cells are then added to the siRNA mixture. Cells are incubated for 24 hours prior to RNA purification. Dose experiments are performed at 10 nM and 0.1 nM final duplex concentration.

Hep3B cells were grown to near confluence at 37°C in an atmosphere of 5% CO₂ in Minimum Essential Medium Alpha (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Transfection was carried out by adding 14.6 μ l of Opti-MEM plus 0.4 μ l of Lipofectamine RNAimax per well to 5 μ l of each siRNA duplex to an individual well in a 96-well plate. The mixture was then incubated at room temperature for 15 minutes. Eighty μ l of complete growth media without antibiotic containing $\sim 1.5 \times 10^4$ cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Dose experiments were performed at 10 nM, 1 nM and 0.1 nM final duplex concentration.

Total RNA isolation using DYNABEADS mRNA Isolation Kit:

RNA was isolated using an automated protocol on a BioTek-EL406 platform using DYNABEADS (Invitrogen, cat#61012). Briefly, 70 μ l of Lysis/Binding Buffer and 10 μ l of lysis buffer containing 3 μ l of magnetic beads were added to the plate with cells. Plates were incubated on an electromagnetic shaker for 10 minutes at room temperature and then magnetic beads were captured and the supernatant was removed. Bead-bound RNA was then washed 2 times with 150 μ l Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150 μ l Elution Buffer, re-captured and supernatant removed.

Synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813):

Ten μ l of a master mix containing 1 μ l 10X Buffer, 0.4 μ l 25X dNTPs, 1 μ l 10x Random primers, 0.5 μ l Reverse Transcriptase, 0.5 μ l RNase inhibitor and 6.6 μ l of H₂O per reaction was added to RNA isolated above. Plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2 h 37°C.

Real time PCR:

Two μ l of cDNA and 5 μ l Lightcycler 480 probe master mix (Roche Cat # 04887301001) were added to either 0.5 μ l of human GAPDH TaqMan Probe and 0.5 μ l human CIDEA probe per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche). Each duplex was tested at least two times and data were normalized to cells transfected with a non-targeting control siRNA. To calculate relative fold change, real time data were analyzed using the $\Delta\Delta C_t$ method and normalized to assays performed with cells transfected with a non-targeting control siRNA.

The results of the screening of the dsRNA agents listed in Table 6 in Hep3B cells are shown in Table 7. An additional single dose (10 nM) screening of the dsRNA agents listed in Table 4 in Hep3B cells was performed. CIDEB signal was normalized to GAPDH. The results are shown in Table 8.

5 Table 7. CIDEB Single Dose Screens in Hep3B Cells

	10 nM Dose		1 nM Dose		0.1 nM Dose	
Duplex ID	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD
AD-1700791.1	88.90	10.50	99.00	15.60	151.20	9.60
AD-1699864.1	92.80	20.20	107.70	22.30	140.30	17.20
AD-1699867.1	27.40	8.30	41.80	6.50	72.30	3.30
AD-1720281.1	77.20	10.40	91.20	14.40	92.70	19.30
AD-1699868.1	41.80	9.30	40.30	12.80	73.30	4.20
AD-1699869.1	63.10	8.70	93.40	1.80	94.50	9.70
AD-1699870.1	54.70	10.50	77.00	15.50	95.70	6.10
AD-1699871.1	32.60	6.70	55.70	7.40	62.00	7.70
AD-1699872.1	49.30	7.50	79.70	18.10	87.40	24.30
AD-1700793.1	44.90	7.40	60.30	6.80	67.40	8.50
AD-1699873.1	42.30	8.10	60.70	5.70	74.90	14.20
AD-1700794.1	78.00	14.40	87.00	8.10	82.70	10.40
AD-1699875.1	47.50	13.10	94.00	11.70	104.60	12.80
AD-1700795.1	73.30	11.30	29.70	9.70	73.80	10.10
AD-1700796.1	58.60	15.10	61.10	10.30	67.40	3.60
AD-1700797.1	62.20	7.50	65.00	15.00	61.30	11.70
AD-1700798.1	29.90	2.50	38.50	13.10	72.50	10.40
AD-1699882.1	42.40	7.40	47.40	11.50	68.00	13.80
AD-1700799.1	40.40	3.40	66.10	11.30	95.40	11.80
AD-1700800.1	46.90	8.60	40.00	9.40	75.00	7.50
AD-1699891.1	55.50	7.50	88.80	7.80	115.80	19.50
AD-1700801.1	54.10	3.90	81.00	15.40	96.80	11.20
AD-1699892.1	66.20	2.70	103.90	15.40	110.00	8.60
AD-1700802.1	19.30	4.60	57.70	8.30	110.90	16.20
AD-1699904.1	36.80	9.60	64.00	11.40	98.10	13.00
AD-1720313.1	80.30	14.70	94.40	20.60	91.30	8.30
AD-1700804.1	43.00	6.20	70.40	21.00	102.60	13.70
AD-1700805.1	36.30	6.40	49.90	4.20	83.20	14.30
AD-1700806.1	7.00	2.20	39.90	7.30	79.20	15.20
AD-1699907.1	18.00	4.20	48.80	12.30	94.00	11.30
AD-1700807.1	10.80	1.90	51.50	5.70	98.40	3.80
AD-1700808.1	31.30	3.20	70.10	15.30	94.90	16.40
AD-1700809.1	34.00	3.90	92.20	13.00	113.70	4.20

Duplex ID	10 nM Dose		1 nM Dose		0.1 nM Dose	
	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD
AD-1700810.1	15.60	1.10	25.70	3.00	99.20	7.70
AD-1700811.1	17.30	2.80	37.20	7.40	78.00	10.10
AD-1700812.1	35.90	5.40	38.10	10.60	33.40	0.30
AD-1700813.1	2.20	0.70	7.90	1.40	59.20	12.30
AD-1700814.1	11.70	2.00	32.60	6.90	110.80	15.90
AD-1700815.1	21.00	4.60	27.10	5.80	58.50	12.30
AD-1700816.1	5.10	1.10	20.70	2.60	87.20	13.70
AD-1700817.1	7.70	0.60	38.60	6.40	106.50	8.40
AD-1699948.1	7.30	0.50	13.90	2.90	99.50	12.00
AD-1699949.1	5.30	0.60	24.20	3.10	89.60	8.60
AD-1720317.1	3.70	1.10	12.20	2.30	98.60	7.60
AD-1700819.1	3.40	0.80	27.70	6.70	62.90	9.10
AD-1699951.1	7.80	1.40	12.50	3.90	37.60	2.60
AD-1720315.1	3.60	1.60	7.70	2.60	40.10	6.20
AD-1700821.1	4.70	1.10	10.80	1.00	71.90	10.20
AD-1699964.1	3.50	0.70	6.50	2.30	45.90	11.40
AD-1700822.1	59.70	12.40	58.70	12.40	78.20	14.00
AD-1699967.1	3.60	1.20	14.80	2.10	100.00	12.10
AD-1720377.1	4.00	1.50	38.70	2.60	82.10	12.20
AD-1699970.1	3.90	1.00	7.70	0.80	54.70	10.30
AD-1699971.1	3.50	1.80	7.60	2.00	51.80	1.60
AD-1699972.1	8.00	1.20	44.80	5.80	106.00	7.20
AD-1699973.1	6.00	1.70	16.20	4.20	102.10	14.00
AD-1700824.1	56.40	12.10	40.80	13.00	75.10	14.70
AD-1700825.1	5.00	1.90	17.40	3.70	41.50	23.90
AD-1700826.1	11.70	3.60	22.20	4.60	30.80	8.80
AD-1699976.1	6.20	1.20	7.70	1.10	23.60	4.70
AD-1699977.1	10.80	1.80	37.10	3.60	86.20	20.00
AD-1699978.1	9.60	1.60	31.00	6.60	105.90	13.10
AD-1699979.1	14.40	3.30	37.10	3.20	116.60	19.40
AD-1700828.1	16.10	2.70	27.50	2.90	129.30	10.40
AD-1700829.1	8.90	2.80	15.80	3.40	68.10	4.80
AD-1699982.1	5.50	0.70	15.20	4.50	84.10	10.60
AD-1699983.1	25.10	7.80	85.60	8.80	99.40	15.50
AD-1699984.1	29.70	1.60	37.00	15.20	95.70	14.50
AD-1699985.1	4.60	0.40	14.20	2.80	68.10	11.90
AD-1699986.1	34.00	4.20	48.70	11.30	111.80	15.60
AD-1700830.1	51.30	8.70	62.90	7.00	NA	NA
AD-1699987.1	30.60	6.50	52.60	8.90	44.50	16.50

	10 nM Dose		1 nM Dose		0.1 nM Dose	
Duplex ID	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD
AD-1699988.1	14.30	1.80	53.90	5.90	95.90	2.20
AD-1700831.1	57.20	8.20	96.80	5.40	124.70	15.30
AD-1699989.1	5.30	0.70	47.50	6.00	101.10	15.20
AD-1699990.1	39.80	11.40	41.50	10.10	88.30	9.30
AD-1700832.1	64.90	16.10	47.00	19.10	83.30	2.90
AD-1700833.1	19.90	3.70	27.60	1.10	81.00	8.50
AD-1700834.1	27.80	3.90	52.90	8.40	51.00	12.80
AD-1700015.1	7.50	2.40	19.60	1.70	93.20	28.00
AD-1700035.1	17.30	2.20	35.20	7.10	63.30	27.50
AD-1700037.1	79.80	21.40	79.30	10.30	121.70	24.40
AD-1700038.1	14.60	1.90	34.80	5.50	71.00	2.50
AD-1700039.1	13.40	2.80	21.20	1.00	66.60	12.70
AD-1700837.1	27.10	8.60	44.20	6.10	96.10	12.00
AD-1700040.1	18.30	3.90	45.90	8.20	80.60	17.60
AD-1700041.1	85.20	12.40	57.70	9.70	93.80	4.80
AD-1700838.1	20.60	6.60	53.80	6.90	114.40	34.60
AD-1700839.1	35.10	2.40	61.20	5.90	97.90	10.40
AD-1700840.1	29.50	1.40	57.10	12.40	88.60	8.60
AD-1700077.1	14.00	1.70	29.60	8.30	87.70	16.40
AD-1700841.1	7.10	1.00	21.90	5.20	105.40	13.10
AD-1700842.1	9.30	1.50	42.90	10.00	117.30	6.70
AD-1700080.1	17.10	1.30	72.40	6.50	62.60	17.90
AD-1700081.1	16.20	6.70	63.30	8.60	104.20	18.00
AD-1700082.1	6.50	2.20	16.60	3.60	67.10	6.00
AD-1700083.1	9.70	1.60	30.30	6.10	91.20	13.40
AD-1700843.1	38.00	11.60	61.70	6.20	54.70	16.90
AD-1700844.1	3.10	1.60	14.20	3.40	75.40	3.70
AD-1700091.1	13.40	0.60	31.60	4.60	68.90	5.00
AD-1700845.1	16.10	5.30	54.50	11.00	49.70	11.80
AD-1700092.1	5.80	0.60	24.80	1.10	93.80	18.00
AD-1700846.1	3.90	0.30	36.70	14.00	118.60	34.70
AD-1700094.1	5.00	1.00	22.30	2.60	88.70	29.10
AD-1700848.1	3.60	1.10	22.10	3.20	99.30	8.80
AD-1700097.1	10.10	2.30	34.30	10.90	85.90	12.50
AD-1720295.1	65.40	5.40	86.20	6.30	102.10	15.80
AD-1700850.1	21.90	7.20	75.90	7.50	86.30	14.20
AD-1700851.1	35.20	6.20	81.10	18.50	78.60	29.30
AD-1700099.1	17.40	3.90	29.40	12.60	42.00	18.00
AD-1700100.1	14.00	2.40	60.00	1.50	110.20	4.40

Duplex ID	10 nM Dose		1 nM Dose		0.1 nM Dose	
	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD
AD-1700101.1	28.70	8.00	98.10	12.00	123.70	13.20
AD-1700852.1	19.70	2.30	71.30	7.30	106.20	14.50
AD-1720289.1	14.40	3.90	46.30	3.50	41.40	2.30
AD-1700853.1	14.40	1.70	90.70	7.00	116.20	15.10
AD-1700854.1	27.20	6.90	62.20	8.80	81.90	10.10
AD-1700104.1	31.50	28.20	49.30	11.40	98.00	9.90
AD-1700855.1	3.30	1.10	11.70	2.20	84.40	19.20
AD-1700856.1	7.80	1.10	29.30	5.00	69.60	6.90
AD-1700109.1	13.60	3.70	37.10	7.90	82.80	15.90
AD-1720370.1	4.00	0.60	13.70	4.40	80.70	15.10
AD-1700858.1	14.70	1.30	74.20	6.60	106.00	3.90
AD-1700859.1	7.70	3.00	32.10	12.30	99.40	24.30
AD-1700860.1	12.70	1.60	64.00	2.20	100.90	1.40
AD-1700112.1	8.70	1.30	20.30	2.90	107.30	11.10
AD-1700113.1	16.60	4.20	63.60	4.10	77.70	10.50
AD-1720300.1	27.30	7.70	81.20	12.70	82.30	21.90
AD-1700114.1	15.10	3.30	37.90	6.00	101.70	8.00
AD-1720286.1	9.10	2.80	25.40	5.00	103.60	10.80
AD-1720287.1	31.10	3.70	74.40	20.90	116.20	21.00
AD-1720288.1	6.30	1.10	34.40	1.60	119.50	5.00
AD-1700115.1	24.50	2.30	70.80	9.20	90.30	29.30
AD-1700863.1	23.20	9.00	56.20	7.00	106.00	10.00
AD-1700116.1	15.70	2.40	47.40	6.60	71.10	11.70
AD-1700864.1	33.10	1.50	79.20	28.00	69.70	15.30
AD-1700865.1	12.00	1.90	41.00	7.60	89.90	6.80
AD-1700121.1	35.50	6.10	92.50	16.90	113.60	16.00
AD-1720311.1	38.70	2.40	61.80	3.00	104.60	11.90
AD-1700122.1	46.30	6.30	40.00	11.20	83.10	9.50
AD-1700867.1	23.70	4.00	38.40	11.00	80.80	12.10
AD-1700125.1	10.90	1.60	42.70	10.50	92.70	7.30
AD-1700868.1	43.00	3.60	85.80	13.80	80.60	17.10
AD-1700869.1	14.20	3.20	47.50	11.00	67.80	6.70
AD-1700870.1	4.00	1.10	28.00	7.20	75.90	14.00
AD-1700871.1	2.00	0.20	13.80	3.70	77.60	6.90
AD-1700872.1	5.30	1.30	29.50	7.60	68.50	14.00
AD-1700873.1	24.00	5.40	49.20	4.60	80.50	12.60
AD-1700874.1	67.90	11.70	95.50	18.20	115.20	7.10
AD-1700875.1	11.20	3.10	45.80	29.40	100.30	5.40
AD-1700876.1	35.20	11.40	83.30	8.90	108.00	17.90

Duplex ID	10 nM Dose		1 nM Dose		0.1 nM Dose	
	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD
AD-1720298.1	13.70	2.30	47.80	4.50	129.30	28.70
AD-1700877.1	17.70	2.60	78.30	6.40	95.40	12.00
AD-1700148.1	5.40	1.10	22.50	5.00	85.10	8.40
AD-1700149.1	23.20	9.10	51.70	9.30	45.80	15.30
AD-1700878.1	8.90	1.60	57.70	9.30	86.10	11.20
AD-1700879.1	72.70	12.80	74.60	26.30	90.30	15.10
AD-1700880.1	7.70	1.90	27.60	7.70	67.50	6.50
AD-1700881.1	2.60	1.10	9.70	2.30	97.60	13.30
AD-1700882.1	6.50	1.20	23.50	7.20	88.60	19.70
AD-1700883.1	4.30	0.40	20.30	4.80	97.00	15.50
AD-1700156.1	5.20	1.00	18.50	4.10	66.10	8.80
AD-1720282.1	7.20	1.30	12.80	1.00	96.00	5.10
AD-1700885.1	14.20	2.20	45.70	5.20	95.30	8.10
AD-1700158.1	6.20	0.90	29.90	3.50	99.10	7.20
AD-1700159.1	5.20	1.50	18.30	2.70	84.30	11.90
AD-1700160.1	5.40	1.50	19.20	4.20	119.70	13.70
AD-1700887.1	29.30	6.00	51.90	5.70	78.50	11.50
AD-1700888.1	6.70	1.10	37.80	6.30	94.20	11.10
AD-1700889.1	26.40	4.20	77.90	26.00	83.60	11.10
AD-1700166.1	35.00	5.80	109.20	19.10	114.50	16.10
AD-1700167.1	41.80	12.40	78.70	11.50	75.20	9.70
AD-1700891.1	34.90	2.00	97.10	6.20	90.10	15.60
AD-1700892.1	74.40	8.70	84.00	22.10	84.70	9.20
AD-1700893.1	6.40	2.50	29.60	4.80	82.20	3.80
AD-1700894.1	30.60	1.60	84.70	25.00	99.90	6.10
AD-1700180.1	6.00	0.90	27.30	1.70	59.90	8.50
AD-1700895.1	4.10	0.90	26.20	5.80	98.40	21.30
AD-1700187.1	7.70	1.30	30.70	6.80	90.60	11.90
AD-1720299.1	13.70	2.10	54.40	3.90	85.40	12.50
AD-1700188.1	17.30	4.60	47.70	14.40	66.40	16.60
AD-1700897.1	55.90	10.80	99.50	8.20	74.20	4.10
AD-1700898.1	93.60	13.90	110.20	24.00	109.30	12.20
AD-1700189.1	52.60	7.80	77.40	13.30	93.30	7.40
AD-1700190.1	8.50	2.20	51.00	3.20	72.50	10.90
AD-1700191.1	4.70	0.40	16.30	2.80	102.90	5.30
AD-1700899.1	29.20	1.80	36.80	4.80	97.30	7.20
AD-1700193.1	16.60	2.20	57.60	10.60	106.70	5.90
AD-1700900.1	46.40	4.40	73.10	9.30	86.10	9.80
AD-1700213.1	8.50	1.10	71.30	4.20	102.50	17.40

	10 nM Dose		1 nM Dose		0.1 nM Dose	
Duplex ID	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD
AD-1720310.1	8.60	1.60	39.20	7.10	130.30	5.30
AD-1700902.1	8.10	4.30	45.70	4.30	111.90	15.50
AD-1700903.1	16.90	3.30	68.70	15.00	96.10	6.40
AD-1700904.1	15.60	2.30	36.60	6.90	79.30	9.10
AD-1700905.1	7.70	1.70	19.50	2.30	50.80	17.60
AD-1700242.1	8.80	1.00	30.90	4.40	109.10	9.10
AD-1700243.1	24.70	5.30	66.40	7.90	65.50	19.60
AD-1700244.1	11.50	0.90	46.60	6.80	84.90	4.00
AD-1700906.1	86.90	6.40	97.90	10.00	98.40	5.00
AD-1700245.1	49.20	3.70	89.60	8.20	116.20	5.10
AD-1700907.1	34.70	12.20	87.10	23.80	96.70	15.50
AD-1700908.1	63.10	11.60	91.30	13.60	99.30	20.20
AD-1700909.1	59.00	9.20	96.30	8.70	69.00	11.00
AD-1700248.1	26.70	7.90	59.50	10.10	89.30	13.50
AD-1700249.1	45.10	4.50	90.70	4.50	NA	NA
AD-1720301.1	52.70	8.10	85.10	11.10	72.90	16.20
AD-1720302.1	58.90	15.30	68.90	9.00	71.30	21.50
AD-1720303.1	40.30	7.70	79.90	12.30	79.40	21.50
AD-1700250.1	26.10	4.80	75.80	10.10	102.40	16.20
AD-1720379.1	47.20	10.00	97.90	19.90	117.60	12.20
AD-1700912.1	84.60	10.20	86.70	10.50	86.40	20.90
AD-1700913.1	82.20	14.30	105.20	16.40	120.70	10.30
AD-1700252.1	17.90	1.50	60.80	8.90	116.20	14.10
AD-1720381.1	45.80	7.20	70.20	6.30	108.00	15.70
AD-1700272.1	34.00	4.60	86.00	9.90	115.40	20.90
AD-1700915.1	5.00	0.70	14.10	3.80	68.60	8.30
AD-1700279.1	16.50	2.50	60.80	5.10	90.10	13.30
AD-1700280.1	8.00	3.70	25.60	5.90	89.00	10.80
AD-1700916.1	7.10	2.70	23.20	5.90	69.30	14.50
AD-1700917.1	17.60	3.70	25.30	7.90	65.70	8.40
AD-1700918.1	27.80	8.10	51.10	11.90	102.80	5.10
AD-1700303.1	4.70	1.70	13.60	4.60	79.00	15.00
AD-1700919.1	8.50	1.30	47.50	6.90	104.70	9.70
AD-1700920.1	14.10	2.30	24.40	4.60	66.30	18.10
AD-1700309.1	4.30	0.70	17.50	5.70	43.00	6.40
AD-1720375.1	5.90	1.60	14.80	7.20	29.50	5.80
AD-1700922.1	8.60	0.60	29.70	6.10	80.70	12.00
AD-1700923.1	6.10	1.30	23.60	6.30	46.90	25.40
AD-1700312.1	2.70	0.50	6.90	2.50	54.70	5.50

	10 nM Dose		1 nM Dose		0.1 nM Dose	
Duplex ID	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD
AD-1700924.1	32.60	13.00	57.40	10.30	101.80	15.30
AD-1700925.1	3.10	0.80	9.90	1.50	62.30	8.20
AD-1700314.1	3.40	1.00	3.80	1.40	37.20	11.50
AD-1720309.1	5.10	0.50	12.70	1.40	37.50	21.80
AD-1700315.1	5.30	1.40	11.80	1.00	62.10	11.00
AD-1720382.1	6.30	1.40	14.50	1.80	74.60	7.90
AD-1720383.1	8.70	5.10	28.60	3.20	92.20	3.20
AD-1720384.1	9.10	4.00	11.30	2.30	45.70	4.60
AD-1700316.1	8.80	1.20	38.50	6.20	118.30	5.00
AD-1700317.1	6.10	1.00	17.60	1.70	87.40	5.60
AD-1700318.1	2.20	0.50	10.80	6.10	55.00	12.00
AD-1700929.1	10.10	2.10	41.20	4.60	80.00	7.60
AD-1700930.1	5.00	1.80	9.50	3.20	91.80	8.60
AD-1700931.1	26.40	3.10	44.80	4.30	76.50	5.80
AD-1700323.1	3.60	0.40	9.10	2.20	85.70	8.30
AD-1720283.1	4.40	1.00	10.40	2.90	66.70	3.50
AD-1700324.1	5.50	2.50	10.10	3.00	71.20	9.60
AD-1720280.1	19.30	3.30	14.40	6.30	81.60	7.10
AD-1700325.1	18.40	2.00	66.70	13.20	113.10	9.10
AD-1700327.1	4.00	0.90	40.20	9.10	98.90	18.00
AD-1700328.1	15.60	1.90	64.90	11.20	92.40	14.80
AD-1700329.1	3.00	1.60	29.10	1.50	114.60	3.90
AD-1700934.1	51.80	3.60	101.90	18.00	104.70	7.20
AD-1700336.1	12.10	2.80	41.70	7.80	92.70	10.50
AD-1720296.1	62.10	11.20	96.90	14.60	84.90	19.00
AD-1700936.1	51.10	7.00	112.80	5.70	94.20	11.60
AD-1700937.1	72.00	17.20	108.40	6.20	134.20	13.90
AD-1700339.1	11.40	3.40	32.70	7.90	49.00	16.70
AD-1700938.1	6.90	1.60	39.40	4.40	82.60	7.60
AD-1700939.1	85.80	10.00	81.50	20.20	103.20	26.50
AD-1700940.1	65.80	14.30	99.20	19.70	90.40	15.30
AD-1700344.1	9.30	1.20	40.60	4.10	99.40	3.90
AD-1720368.1	9.20	2.10	36.20	2.60	101.10	15.10
AD-1700942.1	35.00	8.20	89.10	21.90	100.00	26.10
AD-1700943.1	5.30	1.40	23.30	7.20	53.90	1.30
AD-1700944.1	5.70	1.20	34.60	2.70	70.60	6.30
AD-1720284.1	4.90	1.20	20.30	1.50	104.40	12.90
AD-1700350.1	31.40	4.90	70.00	4.80	117.60	13.90
AD-1700352.1	3.30	0.80	8.90	1.20	116.20	12.30

Duplex ID	10 nM Dose		1 nM Dose		0.1 nM Dose	
	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD
AD-1700353.1	12.70	5.10	19.60	3.30	57.00	7.80
AD-1700947.1	5.60	0.30	17.50	5.40	86.00	14.10
AD-1700354.1	2.30	0.30	4.00	1.50	69.50	6.70
AD-1700355.1	5.00	1.10	8.80	1.00	69.30	12.30
AD-1700356.1	16.80	2.40	53.10	8.20	NA	NA
AD-1700357.1	22.00	2.00	72.30	7.70	79.60	14.10
AD-1700358.1	2.90	0.30	19.90	2.60	75.20	3.00
AD-1700359.1	16.70	2.60	50.60	19.70	112.30	19.00
AD-1700948.1	9.70	3.00	31.70	9.50	79.90	13.70
AD-1700949.1	16.30	1.30	40.90	1.30	60.30	6.70
AD-1700950.1	16.90	7.00	35.70	10.10	82.60	18.60
AD-1700365.1	3.30	0.40	17.10	3.40	72.60	11.90
AD-1700952.1	53.20	9.20	87.00	25.10	83.30	16.80
AD-1700366.1	4.20	0.40	41.80	12.00	91.30	18.00
AD-1700953.1	24.50	6.40	64.70	12.30	71.10	8.60
AD-1720293.1	34.70	7.10	13.20	0.40	61.70	25.00
AD-1700368.1	8.90	2.00	32.80	4.00	86.50	5.70
AD-1700955.1	22.40	0.80	68.50	11.50	77.60	15.50
AD-1700369.1	3.90	1.80	25.60	3.70	80.40	10.10
AD-1700370.1	37.10	3.80	43.90	19.10	94.50	10.70
AD-1700371.1	6.70	2.30	21.00	2.90	114.80	4.20
AD-1700957.1	49.80	8.40	84.60	18.90	92.50	26.30
AD-1700372.1	6.60	0.60	27.70	5.80	95.70	17.40
AD-1700374.1	3.50	0.40	11.50	0.60	38.20	8.50
AD-1700958.1	53.10	2.30	100.80	10.40	89.10	11.80
AD-1700959.1	38.60	0.50	71.60	3.90	92.20	6.30
AD-1720285.1	23.80	7.00	47.20	9.00	85.30	23.20
AD-1700375.1	4.60	1.10	30.30	6.40	93.00	16.90
AD-1700376.1	6.10	1.10	19.30	7.60	76.10	6.00
AD-1700378.1	3.10	1.20	27.10	8.40	79.90	13.10
AD-1700960.1	26.70	5.10	38.50	11.60	61.00	3.70
AD-1700961.1	4.30	0.80	11.90	1.30	116.60	6.70
AD-1700962.1	51.00	10.60	30.90	3.10	71.80	22.90
AD-1700963.1	3.40	0.20	28.70	4.40	99.00	8.10
AD-1700383.1	4.60	1.10	11.10	6.40	69.10	10.20
AD-1720385.1	4.00	0.70	16.50	4.40	89.10	4.50
AD-1700965.1	12.20	4.20	16.50	0.60	44.30	7.90
AD-1700384.1	5.30	1.60	11.60	5.40	53.40	20.40
AD-1700385.1	3.10	0.60	11.40	4.40	88.40	10.70

Duplex ID	10 nM Dose		1 nM Dose		0.1 nM Dose	
	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD
AD-1700386.1	6.10	0.80	10.70	5.10	47.80	19.00
AD-1700387.1	11.70	2.40	37.20	6.20	135.50	4.40
AD-1720380.1	5.50	1.30	12.80	1.80	100.60	18.50
AD-1700967.1	4.40	0.80	20.60	5.10	68.30	10.90
AD-1700388.1	3.80	0.30	17.60	4.30	71.10	20.40
AD-1700968.1	6.10	2.20	9.30	3.70	57.80	14.10
AD-1700969.1	5.40	1.80	20.60	1.00	94.80	12.70
AD-1700408.1	1.80	0.50	5.80	2.00	54.20	10.80
AD-1700970.1	16.10	3.10	54.70	10.10	105.40	13.00
AD-1700971.1	34.30	8.70	82.40	15.30	107.40	15.10
AD-1700410.1	2.50	0.20	12.20	3.80	95.60	18.30
AD-1700411.1	8.20	2.20	21.70	7.50	63.60	14.90
AD-1720304.1	6.30	2.60	14.30	7.70	76.30	12.80
AD-1700973.1	16.10	6.40	64.00	3.00	45.20	12.20
AD-1700413.1	25.70	6.40	83.40	5.30	129.00	8.50
AD-1700414.1	7.20	1.30	45.20	9.60	125.30	17.80
AD-1700975.1	17.50	5.80	50.20	7.50	101.50	16.30
AD-1700976.1	8.80	1.60	32.00	8.10	97.10	6.90
AD-1700977.1	10.70	1.70	26.60	5.00	48.90	4.90
AD-1700978.1	15.30	8.80	21.50	5.00	80.20	13.10
AD-1700422.1	31.50	6.10	62.90	9.00	96.10	4.30
AD-1720305.1	57.00	8.60	73.70	10.10	120.40	6.50
AD-1700423.1	27.10	6.00	27.10	8.50	71.10	26.30
AD-1700424.1	4.00	0.40	15.90	1.80	106.10	16.00
AD-1700425.1	6.50	0.80	43.50	9.50	110.30	8.90
AD-1700426.1	29.00	5.00	80.60	15.50	84.50	21.00
AD-1700427.1	30.30	6.20	49.40	28.80	103.70	7.20
AD-1700980.1	28.90	5.80	57.20	5.30	68.90	13.30
AD-1700428.1	10.00	1.30	37.70	8.40	66.20	18.80
AD-1700981.1	70.90	17.00	89.10	27.90	89.60	12.40
AD-1700982.1	11.70	5.90	36.50	3.40	93.70	21.90
AD-1700439.1	5.30	2.60	38.90	10.50	112.10	8.30
AD-1720316.1	33.30	5.70	66.20	7.50	90.90	11.00
AD-1700984.1	56.50	12.40	98.20	31.40	114.50	13.90
AD-1700985.1	9.20	3.50	41.30	9.80	92.40	9.20
AD-1700461.1	2.90	0.30	6.50	1.50	72.20	18.30
AD-1700462.1	40.30	6.00	92.30	11.20	59.00	6.30
AD-1700986.1	93.90	18.50	105.10	29.10	103.00	24.00
AD-1700464.1	18.80	3.60	48.90	9.50	109.00	10.90

	10 nM Dose		1 nM Dose		0.1 nM Dose	
Duplex ID	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD
AD-1700465.1	34.70	5.80	84.10	8.60	106.50	18.90
AD-1700468.1	10.40	0.90	54.10	9.10	64.80	13.00
AD-1720378.1	11.50	1.30	72.70	3.80	90.40	13.60
AD-1700988.1	10.70	1.50	34.50	7.80	56.30	20.30
AD-1700989.1	73.60	18.20	92.30	9.60	93.60	12.60
AD-1700990.1	2.40	0.60	7.50	1.00	72.10	11.90
AD-1700473.1	3.30	0.90	7.40	0.90	50.10	5.40
AD-1700991.1	3.20	1.10	9.20	1.60	100.50	8.00
AD-1700474.1	4.90	0.50	23.90	4.30	91.60	5.30
AD-1700476.1	2.30	0.80	5.60	0.60	43.50	5.60
AD-1700992.1	92.10	8.00	107.60	10.00	85.50	17.40
AD-1700477.1	12.70	1.50	65.60	9.30	83.00	9.30
AD-1700479.1	3.50	0.80	17.40	0.80	68.80	11.00
AD-1720373.1	3.10	1.00	11.10	3.30	58.70	12.70
AD-1700482.1	7.50	1.50	38.40	5.20	91.20	6.30
AD-1720291.1	25.70	6.90	24.20	1.80	80.90	24.50
AD-1700995.1	3.10	0.90	5.20	1.80	67.00	15.40
AD-1700485.1	16.40	1.20	36.80	4.10	103.00	14.40
AD-1720306.1	30.40	5.50	34.90	7.10	87.90	10.40
AD-1720307.1	55.10	15.00	71.40	4.10	102.90	9.10
AD-1720308.1	4.70	0.50	10.10	1.40	66.10	5.00
AD-1700486.1	75.60	8.60	66.70	28.30	78.20	19.50
AD-1700487.1	6.80	1.70	10.30	3.20	58.70	8.50
AD-1700488.1	15.60	2.00	51.30	15.20	111.30	23.70
AD-1700489.1	51.30	2.50	83.20	12.30	73.60	5.50
AD-1700997.1	13.30	2.40	93.60	9.10	75.40	19.50
AD-1700490.1	10.50	2.00	70.70	9.00	89.50	11.40
AD-1700998.1	66.00	11.40	64.00	8.40	70.00	10.70
AD-1700517.1	25.80	5.30	61.30	5.90	84.70	11.60
AD-1700519.1	48.60	13.10	84.20	39.10	111.20	10.60
AD-1720292.1	76.80	8.50	70.20	2.80	74.50	12.10
AD-1700520.1	12.80	3.00	46.20	8.80	85.00	10.20
AD-1720376.1	50.40	10.70	79.60	4.60	93.70	7.40
AD-1701001.1	53.90	6.70	88.90	7.10	115.50	27.10
AD-1701002.1	79.10	10.40	110.00	19.60	111.90	8.60
AD-1700522.1	71.90	7.70	94.40	22.70	115.00	17.00
AD-1701003.1	6.60	1.20	67.80	9.00	90.70	14.20
AD-1700542.1	6.30	0.90	30.00	7.00	101.50	13.60
AD-1701004.1	8.00	3.50	17.30	3.40	76.60	10.50

Duplex ID	10 nM Dose		1 nM Dose		0.1 nM Dose	
	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD
AD-1701005.1	3.50	1.50	16.00	3.90	90.10	10.00
AD-1700544.1	3.60	0.70	11.60	3.80	84.10	9.30
AD-1700550.1	4.10	1.90	4.80	2.20	46.60	6.90
AD-1720386.1	6.20	1.70	11.60	5.40	64.50	9.00
AD-1701007.1	11.30	2.10	11.00	3.70	42.10	5.90
AD-1700553.1	3.80	0.70	6.00	2.30	77.80	6.40
AD-1700554.1	4.80	0.90	6.60	1.20	73.50	17.60
AD-1700555.1	5.10	1.20	6.40	1.00	63.80	9.50
AD-1700556.1	4.40	1.50	10.70	1.80	61.00	10.00
AD-1700557.1	5.10	2.60	23.10	7.50	95.90	9.40
AD-1700558.1	5.90	0.80	6.60	1.40	63.40	12.50
AD-1701009.1	18.30	3.10	46.80	5.00	99.80	6.40
AD-1700560.1	6.20	4.60	17.50	4.90	63.90	3.30
AD-1720372.1	3.20	1.00	7.30	1.30	56.50	14.90
AD-1701011.1	3.10	0.90	18.50	2.70	75.10	7.90
AD-1701012.1	7.50	2.60	15.00	2.80	78.00	13.90
AD-1701013.1	27.60	5.60	68.10	10.00	115.30	30.40
AD-1700563.1	8.20	1.50	29.30	4.50	114.10	11.00
AD-1701014.1	8.40	0.60	29.90	2.60	83.40	10.10
AD-1700566.1	3.00	1.30	8.10	1.60	82.30	8.40
AD-1720312.1	5.80	3.20	17.10	2.10	108.80	7.00
AD-1701016.1	4.70	0.20	14.90	2.40	83.40	12.00
AD-1701017.1	9.30	1.80	26.60	3.50	115.80	5.30
AD-1700573.1	2.80	1.70	12.70	2.20	67.70	8.00
AD-1720371.1	7.90	0.80	12.40	2.00	74.20	10.60
AD-1701019.1	7.50	2.40	18.30	2.90	86.50	14.90
AD-1700575.1	9.70	1.80	18.90	2.60	106.30	9.00
AD-1700576.1	31.80	6.50	61.00	5.90	84.80	8.00
AD-1701020.1	7.00	1.50	41.80	5.50	105.50	10.20
AD-1700578.1	9.20	2.00	18.80	3.70	96.70	3.20
AD-1720290.1	5.40	1.50	19.00	3.20	95.50	1.80
AD-1701022.1	5.00	0.30	14.60	2.00	68.70	11.30
AD-1701023.1	10.20	1.40	36.00	9.10	107.40	12.00
AD-1700580.1	7.60	2.10	33.10	6.10	107.50	11.70
AD-1701024.1	12.10	5.90	39.70	10.90	91.00	8.00
AD-1700582.1	8.20	1.30	14.80	3.80	56.90	7.40
AD-1720294.1	5.40	1.20	14.10	2.80	63.40	14.00
AD-1701026.1	3.20	0.50	28.60	3.40	84.40	8.70
AD-1701027.1	5.20	1.00	27.10	3.30	82.10	8.50

	10 nM Dose		1 nM Dose		0.1 nM Dose	
Duplex ID	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD	Avg % CIDEB mRNA Remaining	SD
AD-1700623.1	5.50	1.20	13.10	5.90	95.90	10.40
AD-1700670.1	42.70	7.00	93.20	12.10	96.70	6.20
AD-1700671.1	62.40	12.30	81.80	11.60	82.40	19.80
AD-1720369.1	21.80	3.80	67.10	11.30	98.80	11.70
AD-1701029.1	13.80	5.60	52.00	16.10	96.50	25.40
AD-1700680.1	20.10	1.00	82.90	10.20	99.20	14.30
AD-1720297.1	79.80	3.70	105.80	12.80	101.50	17.30
AD-1701031.1	16.10	3.90	19.90	2.10	93.50	2.90
AD-1700681.1	8.10	1.70	35.00	5.60	76.20	15.60
AD-1701032.1	22.40	8.30	65.10	19.30	80.30	1.10
AD-1700684.1	10.10	3.80	29.70	5.60	96.90	8.50
AD-1701033.1	98.60	7.10	117.50	19.10	123.90	3.90
AD-1700710.1	93.80	16.60	112.90	16.20	122.60	15.20
AD-1701034.1	93.50	11.40	105.10	3.80	110.30	8.20
AD-1701035.1	18.80	1.00	65.90	10.50	89.30	7.70
AD-1700736.1	9.00	3.10	18.80	5.60	72.10	5.00
AD-1720374.1	45.60	14.20	55.40	12.00	61.70	10.40
AD-1701037.1	37.60	12.00	66.20	11.70	69.90	13.10
AD-1700737.1	17.30	3.70	58.00	8.00	71.60	19.10
AD-1700738.1	11.70	1.60	37.80	8.60	92.00	10.90
AD-1701038.1	23.60	1.40	113.80	9.20	93.10	11.80
AD-1700780.1	3.90	2.20	6.10	1.50	35.40	11.10
AD-1720314.1	3.40	1.00	7.30	2.00	59.20	13.00
AD-1700781.1	3.00	0.70	6.90	1.90	51.30	5.40
AD-1700782.1	5.80	1.60	8.80	2.90	58.30	14.20

Table 8. CIDEB Single Dose Screens in Hep3B Cells

Duplex ID	Avg% CIDEB mRNA Remaining		DuplexID	Avg% CIDEB mRNA Remaining	
	10nM			10nM	
	Mean	SD		Mean	SD
AD-1686813	7.034	0.258	AD-1686057.1	5.373	0.633
AD-1686803.1	7.037	0.549	AD-1686053.1	56.574	3.938
AD-1686794.1	6.698	0.335	AD-1686028.1	46.840	2.177
AD-1686783.1	9.004	0.720	AD-1686016.1	9.940	0.590

Duplex ID	Avg% CIDEB mRNA Remaining		DuplexID	Avg% CIDEB mRNA Remaining	
	10nM			10nM	
	Mean	SD		Mean	SD
AD-1686781.1	26.941	1.075	AD-1686007.1	18.073	1.221
AD-1686770.1	7.394	0.857	AD-1685994.1	9.314	0.297
AD-1686761.1	29.344	3.097	AD-1685983.1	15.368	0.551
AD-1686751.1	75.987	4.650	AD-1685969.1	9.303	0.607
AD-1686747.1	74.602	6.448	AD-1685956.1	24.725	7.404
AD-1686738.1	33.637	1.984	AD-1685935.1	38.364	1.701
AD-1686729.1	24.804	3.052	AD-1685931.1	40.894	1.620
AD-1686720.1	20.335	0.768	AD-1685921.1	48.205	4.328
AD-1686709.1	10.072	0.802	AD-1685912.1	54.202	3.053
AD-1686693.1	15.320	1.441	AD-1685902.1	91.948	4.558
AD-1686680.1	8.115	0.694	AD-1685898.1	98.617	4.497
AD-1686669.1	11.933	1.964	AD-1685889.1	84.955	2.049
AD-1686658.1	9.575	0.485	AD-1685878.1	95.346	1.773
AD-1686649.1	7.356	0.570	AD-1685869.1	87.386	2.693
AD-1686639.1	11.744	0.890	AD-1685856.1	90.274	1.411
AD-1686626.1	9.617	0.753	AD-1685847.1	61.105	2.592
AD-1686616.1	10.909	0.749	AD-1685838.1	83.672	4.357
AD-1686606.1	9.636	0.540	AD-1685826.1	77.305	2.383
AD-1686597.1	7.108	0.308	AD-1685810.1	100.598	6.749
AD-1686585.1	5.715	1.579	AD-1685798.1	81.801	6.236
AD-1686576.1	8.745	0.449	AD-1685788.1	76.643	16.621
AD-1686566.1	7.504	0.442	AD-1685779.1	93.379	8.610
AD-1686565.1	51.547	2.866	AD-1685770.1	94.639	5.122
AD-1686556.1	34.239	2.082	AD-1685744.1	93.333	6.221
AD-1686543.1	50.576	2.174	AD-1685735.1	72.951	5.540
AD-1686540.1	50.093	4.978	AD-1685726.1	92.927	8.552
AD-1686531.1	56.092	3.563	AD-1685717.1	84.588	8.485
AD-1686518.1	10.338	0.356	AD-1685708.1	85.659	6.249
AD-1686508.1	56.285	2.808	AD-1685699.1	93.319	3.803

Duplex ID	Avg% CIDEb mRNA Remaining		DuplexID	Avg% CIDEb mRNA Remaining	
	10nM			10nM	
	Mean	SD		Mean	SD
AD-1686491.1	19.182	0.216	AD-1685689.1	98.857	4.806
AD-1686475.1	52.270	2.123	AD-1685673.1	78.962	3.900
AD-1686466.1	8.819	0.655	AD-1685664.1	96.600	15.224
AD-1686451.1	12.861	0.277	AD-1685648.1	89.253	4.285
AD-1686435.1	20.744	0.806	AD-1685637.1	82.011	1.907
AD-1686426.1	15.031	0.938	AD-1685627.1	91.683	23.711
AD-1686417.1	12.149	0.855	AD-1685614.1	89.157	4.921
AD-1686403.1	15.485	1.878	AD-1685610.1	78.640	8.712
AD-1686394.1	12.687	0.710	AD-1685599.1	83.195	5.391
AD-1686377.1	15.920	0.978	AD-1685587.1	93.563	13.290
AD-1686366.1	17.513	1.255	AD-1685570.1	88.283	6.794
AD-1686354.1	18.012	0.876	AD-1685561.1	88.025	5.340
AD-1686343.1	19.232	1.476	AD-1685549.1	82.656	5.181
AD-1686334.1	24.115	1.100	AD-1685538.1	88.232	3.361
AD-1686319.1	13.232	0.799	AD-1685528.1	92.786	7.624
AD-1686286.1	22.559	0.512	AD-1685512.1	97.641	6.841
AD-1686274.1	29.551	0.741	AD-1685503.1	95.865	17.990
AD-1686265.1	70.105	5.349	AD-1685491.1	94.393	11.080
AD-1686251.1	14.695	1.084	AD-1685469.1	85.615	7.133
AD-1686242.1	4.855	0.629	AD-1685418.1	86.858	2.046
AD-1686225.1	10.479	0.390	AD-1685406.1	76.723	3.400
AD-1686216.1	15.955	1.165	AD-1685388.1	83.950	4.269
AD-1686207.1	10.015	1.533	AD-1685379.1	82.859	3.868
AD-1686197.1	4.724	1.010	AD-1685366.1	85.364	1.718
AD-1686181.1	15.356	0.366	AD-1685284.1	82.852	1.693
AD-1686171.1	19.964	0.716	AD-1685271.1	84.648	3.346
AD-1686159.1	12.973	0.587	AD-1685250.1	86.016	3.414
AD-1686147.1	6.105	1.495	AD-1685240.1	79.332	2.819
AD-1686136.1	5.802	0.945	AD-1685228.1	90.535	6.335

Duplex ID	Avg% CIDEB mRNA Remaining		DuplexID	Avg% CIDEB mRNA Remaining	
	10nM			10nM	
	Mean	SD		Mean	SD
AD-1686126.1	11.135	1.538	AD-1685217.1	80.884	4.373
AD-1686116.1	14.156	3.058	AD-1685207.1	74.123	2.932
AD-1686098.1	22.948	0.996	AD-1685198.1	85.722	6.301
AD-1686086.1	53.797	3.239	AD-1685173.1	83.069	7.260
AD-1686075.1	34.648	2.855	AD-1685156.1	99.596	11.628
AD-1686066.1	54.370	2.364			

Example 3. *In vitro* screening methods

A subset of the duplexes was also assessed by transfection and free uptake in primary human hepatocytes and primary cynomolgus hepatocytes

Cell culture and transfections:

Transfection and free uptake assays were carried out in primary human hepatocyte (PHH, BioIVT) and primary cyno hepatocyte (PCH, BioIVT). Transfection was performed by adding of Opti-MEM plus 0.1 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5 µl of siRNA duplexes per well into a 384-well plate and incubated at room temperature for 15 minutes. 40 µl of in Invitrogro CP media (BioIVT, Cat # Z99029) containing $\sim 10 \times 10^3$ cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Experiments were performed at 10nM, 1nM, 0.1nM and 0.01nM. Free uptake assay was performed similarly to transfection assay without using Liporectamine RNAimax and cells were incubated for 48 hours prior to the RNA purification. Experiments were performed at 250nM, 100nM, 10nM, 1nM.

Total RNA isolation using DYNABEADS mRNA Isolation Kit:

RNA was isolated using an Highres Biosolution integration system using Dynabeads™ mRNA DIRECT™ Purification Kit (Invitrogen™, Catalog No. 61012). Briefly, 70 µL of Lysis/Binding Buffer and 10 µL of lysis buffer containing 3 µL of magnetic beads were added to the plate with cells. Plates were incubated on an electromagnetic shaker for 10 minutes at room temperature and then magnetic beads were captured and the supernatant was removed. Bead-bound RNA was then washed 2 times with 90 µL Wash Buffer A and once with 90 µL Wash Buffer B. Beads were then washed with 90 µL Elution Buffer, re-captured, and supernatant was removed. Complementary DNA (cDNA) was synthesized using High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems™, Catalog

No. 4374967) according to the manufacturer's recommendations. A master mix containing 1 μ L 10X Buffer, 0.4 μ L 25X deoxyribonucleotide triphosphate, 1 μ L 10X Random primers, 0.5 μ L Reverse Transcriptase, 0.5 μ L RNase inhibitor, and 6.6 μ L of H₂O per reaction was added to RNA isolated above. The plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2 hours incubation at 37°C.

CIDEB mRNA levels were quantified by performing RT-qPCR analysis. 2 μ L of cDNA were added to a master mix containing 0.5 μ L of human or cyno GAPDH TaqMan Probe, 0.5 μ L human or cyno CIDEB probe) and 5 μ L Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates. Real time PCR was done in a LightCycler480 Real Time PCR system (Roche). To calculate relative fold change, real-time data were analyzed using the Delta-Delta Threshold Cycle (Relative Quantification) ($\Delta\Delta C_q$ [RQ]) method [Schmittgen and Livak 2008] and were normalized to control assays performed using cells transfected with PBS. For all samples, CIDEB mRNA levels were first normalized to *GAPDH* as a reference gene. Data are expressed as percent of CIDEB mRNA remaining relative to average PBS control and error is expressed as standard deviation (SD), derived from the 4 transfection replicates.

The results of the single dose transfection screens and free uptake screens of the dsRNA agents in PCH is shown in Table 9 and PHH cells are shown in Table 10.

Table 9. Transfection and Free Uptake in Primary Cyno Hepatocytes

Duplex ID	Transfection								Free uptake							
	10 nM		1 nM		0.1 nM		0.01nM		250 nM		100 nM		10 nM		1 nM	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1700826.1	9	2	16	5	17	5	25	6	36	5	47	2	73	11	60	2
AD-1699971.1	2	1	5	1	5	1	8	1	16	5	23	4	31	4	44	5
AD-1720309.1	8	1	17	2	22	3	31	3	36	6	41	5	53	12	68	7
AD-1720382.1	7	1	11	3	12	2	18	3	35	5	30	5	46	10	59	8
AD-1700355.1	6	0	14	1	15	3	22	5	25	5	35	7	42	10	55	2
AD-1720294.1	2	0	4	0	7	1	11	3	28	5	31	3	43	7	52	7
AD-1700411.1	10	1	24	2	41	6	45	7	46	7	55	4	55	2	71	7
AD-1700383.1	6	1	11	2	11	1	16	3	25	2	32	6	50	9	60	10
AD-1720304.1	11	4	19	1	29	5	36	4	40	10	48	9	49	1	58	5

Duplex ID	Transfection								Free uptake							
	10 nM		1 nM		0.1 nM		0.01nM		250 nM		100 nM		10 nM		1 nM	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1700082.1	5	0	13	2	13	2	24	3	27	5	32	7	43	9	54	5
AD-1700872.1	3	1	7	1	6	1	13	2	21	4	35	1	44	4	54	9
AD-1700990.1	4	1	8	2	5	1	13	2	46	14	43	7	139	23	64	7
AD-1700558.1	7	1	17	4	23	4	25	2	35	9	42	3	53	9	64	6
AD-1700782.1	3	1	7	1	7	1	16	2	23	3	26	2	49	2	50	8
AD-1720375.1	15	3	22	4	14	2	27	5	55	5	59	4	69	7	82	5
AD-1700358.1	5	1	13	2	18	2	19	2	30	5	37	6	57	5	44	3
AD-1720314.1	16	5	10	2	9	2	14	2	23	6	37	5	40	8	51	10
AD-1700156.1	5	3	9	1	8	1	16	3	26	6	40	6	49	5	70	9
AD-1699964.1	2	0	5	1	4	1	11	3	16	2	19	2	35	6	49	9
AD-1699976.1	1	0	3	0	6	2	6	1	15	2	18	2	32	2	37	2
AD-1701007.1	7	1	9	1	10	1	16	2	28	4	41	4	56	10	53	2
AD-1700386.1	8	1	13	2	15	2	20	3	24	1	32	4	40	7	54	5
AD-1700995.1	3	1	6	1	5	0	12	1	18	2	27	3	32	3	53	8
AD-1700376.1	4	1	7	2	8	1	11	2	20	1	26	2	47	8	49	3
AD-1700915.1	7	1	14	2	16	1	25	4	37	3	32	7	43	11	54	3
AD-1700384.1	6	1	11	2	12	1	18	3	34	3	43	4	63	9	61	5
AD-1700925.1	8	2	14	3	19	3	26	6	35	8	39	4	61	11	46	0
AD-1700378.1	6	1	15	3	15	3	21	4	32	5	34	6	50	5	60	4
AD-1700870.1	4	1	10	1	14	3	24	4	29	3	36	3	54	6	57	2
AD-1700353.1	13	1	21	1	16	1	35	11	44	4	55	3	63	7	88	3
AD-1720371.1	11	2	22	5	28	8	28	7	38	4	47	3	60	10	63	2
AD-1700374.1	4	0	8	1	9	1	14	2	26	3	33	3	54	7	54	3
AD-1700825.1	12	2	22	3	18	4	27	5	40	7	46	11	58	6	71	11

Duplex ID	Transfection								Free uptake							
	10 nM		1 nM		0.1 nM		0.01nM		250 nM		100 nM		10 nM		1 nM	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1720386.1	3	1	5	1	6	1	10	2	27	2	38	4	53	8	56	6
AD-1720315.1	2	0	4	1	4	1	8	1	14	3	23	2	27	6	34	6
AD-1700159.1	2	0	5	1	5	1	9	2	19	2	26	5	44	3	50	1
AD-1700923.1	7	1	16	2	22	4	24	4	29	1	39	7	57	4	52	7
AD-1700369.1	5	2	6	1	11	2	14	1	20	3	24	5	37	8	39	8
AD-1701016.1	5	1	14	3	12	2	20	0	29	5	33	5	43	7	60	4
AD-1700148.1	3	1	6	1	7	1	12	1	18	4	23	4	25	5	46	4
AD-1700318.1	5	1	9	1	13	4	17	1	26	4	30	5	36	8	50	9
AD-1700781.1	2	1	7	2	11	2	12	3	33	16	29	6	47	4	51	7
AD-1700905.1	10	1	15	2	16	2	22	2	40	7	49	8	54	8	57	5
AD-1720308.1	3	1	8	1	10	1	15	2	21	2	29	4	30	7	60	10
AD-1700916.1	16	3	20	2	19	3	23	3	43	3	54	9	62	11	63	9
AD-1700461.1	5	1	7	2	5	1	13	2	29	3	42	8	52	9	64	8
AD-1700871.1	2	1	6	2	12	3	11	3	16	3	19	4	41	10	46	7
AD-1720373.1	4	1	11	3	14	2	20	1	27	2	29	4	49	4	54	8
AD-1701004.1	4	1	9	0	13	2	18	1	35	5	42	4	63	12	60	8
AD-1700556.1	2	0	5	1	5	0	17	7	21	1	24	2	38	9	50	8
AD-1720283.1	14	5	20	5	16	1	30	3	41	2	49	4	54	2	77	13
AD-1700303.1	5	0	10	3	8	1	15	2	25	7	34	7	31	3	45	9
AD-1720370.1	2	0	4	1	6	1	10	1	19	6	25	5	39	5	51	11
AD-1700573.1	4	1	8	1	12	2	17	2	19	5	28	6	36	6	44	2
AD-1720372.1	3	1	9	1	12	1	16	1	26	3	36	7	48	6	59	4
AD-1700473.1	2	1	4	1	5	1	8	1	14	3	21	2	23	6	40	8
AD-1700821.1	2	0	4	1	3	0	6	1	11	1	15	4	20	1	33	1

Duplex ID	Transfection								Free uptake							
	10 nM		1 nM		0.1 nM		0.01nM		250 nM		100 nM		10 nM		1 nM	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1720384.1	4	0	7	1	7	1	12	2	25	8	31	4	40	6	47	10
AD-1700314.1	5	1	10	2	9	1	16	1	20	4	25	3	41	6	48	2
AD-1700582.1	2	1	4	1	6	1	9	1	21	4	25	2	34	2	48	5
AD-1700385.1	6	1	10	1	9	1	15	3	23	5	30	6	34	7	48	8
AD-1700544.1	2	0	7	1	8	1	15	3	21	5	29	4	43	4	49	8
AD-1699970.1	1	0	2	1	2	0	4	1	11	2	13	3	29	6	31	1
AD-1700967.1	7	1	12	1	16	2	27	7	28	4	37	7	44	8	56	8
AD-1700844.1	2	0	5	0	9	1	13	2	23	4	31	4	53	7	47	5
AD-1700555.1	1	0	4	1	4	1	8	0	12	2	18	5	23	10	36	8
AD-1700880.1	5	2	6	1	10	1	12	1	24	4	41	7	43	12	53	1
AD-1700388.1	7	2	12	2	16	3	20	2	24	4	34	10	35	6	54	8
AD-1700560.1	5	1	15	2	22	3	28	4	31	6	38	3	54	4	59	4
AD-1700550.1	2	0	4	1	4	0	7	1	18	0	22	3	39	6	43	3
AD-1700180.1	1	0	3	1	5	1	10	1	22	4	30	3	43	4	46	4
AD-1700312.1	8	3	11	3	7	1	21	7	25	1	31	2	36	3	49	4
AD-1699985.1	2	0	7	1	10	1	13	3	29	0	33	2	48	7	49	3
AD-1701011.1	5	1	12	2	20	4	23	3	28	3	41	2	57	9	57	6
AD-1700476.1	3	1	8	1	14	2	15	1	23	3	37	9	57	11	57	9
AD-1701022.1	3	0	6	1	6	1	13	2	23	4	30	1	38	3	59	3
AD-1700554.1	3	1	5	1	5	1	14	3	18	3	21	6	41	6	63	7
AD-1699951.1	2	0	4	1	5	1	8	1	12	2	16	2	22	4	34	6
AD-1700408.1	5	1	8	2	12	2	13	4	26	6	33	2	57	9	45	5
AD-1700487.1	4	0	11	2	11	2	21	2	28	6	19	2	43	10	51	1
AD-1700943.1	5	1	9	1	9	0	18	0	33	6	43	2	50	5	68	12

Duplex ID	Transfection								Free uptake							
	10 nM		1 nM		0.1 nM		0.01nM		250 nM		100 nM		10 nM		1 nM	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1700736.1	9	2	18	3	19	3	29	2	49	12	61	9	68	15	84	12
AD-1700813.1	3	1	6	1	9	2	12	2	26	6	31	4	36	8	44	3
AD-1700968.1	4	1	8	1	8	2	13	2	23	4	31	8	54	8	50	7
AD-1700780.1	4	1	3	1	5	4	8	2	9	1	14	3	19	3	31	9
AD-1700324.1	9	2	14	2	11	2	21	6	NA	NA	NA	NA	NA	NA	NA	NA
AD-1700566.1	1	0	4	0	5	1	8	1	15	4	17	1	23	5	34	6
AD-1700354.1	4	1	7	1	9	1	10	1	20	3	23	5	30	6	51	5
AD-1700365.1	8	2	14	3	22	3	29	6	33	7	45	5	69	4	61	7
AD-1700553.1	2	1	4	1	4	1	9	2	13	2	23	4	30	4	38	6

Table 10. Transfection and Free Uptake in Primary Human Hepatocytes

Duplex ID	Transfection								Free Uptake							
	10 nM		1 nM		0.1 nM		0.01nM		250 nM		100 nM		10 nM		1 nM	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1700826.1	13	2	24	3	28	7	32	6	33	3	34	3	77	4	108	22
AD-1699971.1	5	1	9	1	15	3	13	3	7	3	8	2	16	3	50	14
AD-1720309.1	12	0	25	4	42	7	48	6	47	10	58	11	75	9	75	22
AD-1720382.1	8	2	10	3	7	4	14	2	21	2	25	3	38	6	73	8
AD-1700355.1	12	1	17	3	25	5	29	10	28	9	32	8	54	4	98	14
AD-1720294.1	4	1	8	1	13	1	16	2	16	3	15	1	25	4	67	12
AD-1700411.1	19	2	33	4	59	10	59	14	63	4	68	14	94	5	115	15
AD-1700383.1	5	3	4	4	7	4	13	4	15	3	15	3	32	7	75	17
AD-1720304.1	17	3	30	4	41	5	41	7	54	10	60	4	73	7	102	10
AD-1700082.1	13	3	23	5	28	7	28	7	37	8	43	4	61	14	93	5
AD-1700872.1	7	1	11	3	16	2	16	2	25	6	25	3	40	4	77	15

Duplex ID	Transfection								Free Uptake							
	10 nM		1 nM		0.1 nM		0.01nM		250 nM		100 nM		10 nM		1 nM	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1700990.1	4	0	7	2	8	2	8	3	5	2	8	2	18	2	33	8
AD-1700558.1	12	1	21	2	28	3	29	4	32	7	40	5	68	4	100	12
AD-1700782.1	8	1	13	1	17	2	22	3	24	2	22	4	43	3	91	19
AD-1720375.1	16	2	20	1	25	4	27	3	38	7	30	2	55	2	88	3
AD-1700358.1	11	2	22	2	28	2	31	3	37	5	36	2	61	8	82	8
AD-1720314.1	5	2	9	3	11	2	10	2	10	2	11	3	19	7	41	20
AD-1700156.1	6	1	8	1	11	2	10	2	14	5	18	3	32	3	55	8
AD-1699964.1	3	0	8	2	9	2	12	2	7	1	7	1	15	1	54	11
AD-1699976.1	6	2	11	2	12	1	13	1	8	2	10	2	24	4	60	12
AD-1701007.1	15	4	15	4	16	3	17	4	11	2	12	2	24	2	61	4
AD-1700386.1	10	0	18	3	29	4	23	2	27	2	38	8	45	9	81	6
AD-1700995.1	5	1	9	2	9	2	10	2	8	1	11	3	21	4	55	19
AD-1700376.1	6	0	13	2	16	2	15	3	9	2	14	3	32	6	70	8
AD-1700915.1	12	1	19	3	29	8	34	8	34	10	33	7	48	9	96	17
AD-1700384.1	8	2	16	2	18	2	20	2	30	4	36	8	46	8	92	2
AD-1700925.1	18	1	27	7	38	4	46	2	70	16	68	9	82	11	122	8
AD-1700378.1	11	2	19	4	28	2	27	3	34	6	42	9	62	12	97	10
AD-1700870.1	14	2	20	4	31	3	35	4	41	8	37	2	71	10	118	4
AD-1700353.1	18	1	22	5	26	4	29	4	44	5	49	4	64	13	107	1
AD-1720371.1	12	3	21	5	26	2	27	5	26	1	29	7	51	7	75	22
AD-1700374.1	8	1	15	2	18	2	18	1	15	1	18	3	37	7	77	17
AD-1700825.1	15	1	20	4	30	6	37	8	46	4	57	10	75	11	115	19
AD-1720386.1	6	1	10	2	12	2	13	2	16	2	15	2	30	5	76	6
AD-1720315.1	6	1	10	2	15	2	14	6	11	5	14	5	24	7	50	21

Duplex ID	Transfection								Free Uptake							
	10 nM		1 nM		0.1 nM		0.01nM		250 nM		100 nM		10 nM		1 nM	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1700159.1	5	0	12	3	13	4	16	3	15	3	20	3	32	5	91	11
AD-1700923.1	17	3	29	6	39	3	45	10	53	10	61	8	84	9	103	11
AD-1700369.1	5	0	10	1	13	1	17	1	6	1	9	1	26	2	56	16
AD-1701016.1	8	2	13	3	18	6	13	5	22	7	22	4	46	4	92	25
AD-1700148.1	7	3	13	2	18	3	17	4	18	6	17	2	38	7	69	6
AD-1700318.1	13	3	18	3	25	3	28	5	32	7	32	8	52	3	88	3
AD-1700781.1	10	1	18	3	20	2	21	3	25	4	23	3	55	9	96	24
AD-1700905.1	13	4	15	3	17	4	24	3	24	4	38	6	25	12	54	11
AD-1720308.1	10	1	17	3	20	2	23	3	19	3	24	6	42	2	78	6
AD-1700916.1	8	2	12	1	15	2	17	3	21	8	8	3	42	8	67	9
AD-1700461.1	6	2	7	3	7	3	10	4	11	3	11	5	17	5	43	18
AD-1700871.1	9	1	13	2	13	2	15	3	10	2	13	2	36	8	65	8
AD-1720373.1	11	0	18	2	27	2	30	3	25	4	33	6	56	6	90	8
AD-1701004.1	6	1	11	1	14	2	15	2	16	3	15	4	35	6	56	12
AD-1700556.1	5	1	8	1	12	2	15	3	10	1	9	2	24	6	67	4
AD-1720283.1	12	1	14	1	21	2	16	3	33	3	31	2	38	5	78	11
AD-1700303.1	9	2	16	4	20	3	19	5	24	3	28	4	48	2	60	8
AD-1720370.1	9	1	15	2	14	3	17	2	12	2	17	4	38	4	65	10
AD-1700573.1	9	1	15	1	18	2	19	2	16	2	21	3	46	11	85	12
AD-1720372.1	10	2	14	2	20	3	22	3	15	3	21	5	48	6	92	16
AD-1700473.1	5	1	9	3	13	4	15	7	9	2	11	3	23	2	52	16
AD-1700821.1	3	0	5	1	9	2	10	1	4	1	5	1	12	3	33	17
AD-1720384.1	5	1	8	3	11	2	14	1	10	2	11	3	28	6	60	26
AD-1700314.1	6	3	7	3	15	0	21	3	11	2	19	4	39	3	83	14

Duplex ID	Transfection								Free Uptake							
	10 nM		1 nM		0.1 nM		0.01nM		250 nM		100 nM		10 nM		1 nM	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1700582.1	5	1	9	2	15	4	13	2	11	2	13	3	20	5	55	4
AD-1700385.1	9	1	13	2	20	3	21	6	12	5	22	4	42	7	57	9
AD-1700544.1	7	1	11	2	17	4	19	6	10	3	13	3	24	2	67	5
AD-1699970.1	3	1	6	2	8	1	8	1	5	1	5	1	10	4	24	3
AD-1700967.1	12	1	24	4	27	3	25	4	40	8	45	11	67	7	100	9
AD-1700844.1	9	1	16	2	21	1	24	1	24	6	28	7	57	8	98	10
AD-1700555.1	4	1	8	1	11	1	9	2	4	1	6	2	12	1	22	8
AD-1700880.1	5	2	10	2	12	4	10	4	9	3	19	4	35	3	42	13
AD-1700388.1	10	0	16	4	27	6	25	2	30	2	36	4	55	8	87	17
AD-1700560.1	11	2	16	2	23	2	25	2	28	5	35	8	61	11	95	9
AD-1700550.1	4	0	10	1	10	1	12	1	7	1	8	2	16	1	51	15
AD-1700180.1	7	1	11	1	16	2	16	1	18	2	14	2	33	1	64	12
AD-1700312.1	5	1	9	1	14	2	12	2	10	3	11	0	27	5	71	10
AD-1699985.1	8	1	20	4	22	4	26	5	31	9	41	9	62	5	105	9
AD-1701011.1	9	1	19	1	23	3	24	3	26	4	31	4	53	3	88	11
AD-1700476.1	12	4	18	2	27	6	31	9	41	1	45	4	59	13	109	7
AD-1701022.1	6	1	10	4	14	2	14	1	15	2	13	2	29	4	69	5
AD-1700554.1	4	1	7	1	6	3	10	2	4	1	3	1	10	3	23	11
AD-1699951.1	6	1	10	2	17	4	13	6	13	4	13	6	26	3	48	5
AD-1700408.1	9	3	15	5	22	2	22	4	29	4	31	6	53	10	95	10
AD-1700487.1	6	2	11	3	14	3	13	5	20	6	27	6	37	11	51	12
AD-1700943.1	8	1	13	2	16	3	21	8	28	4	31	4	53	3	105	17
AD-1700736.1	12	1	17	3	24	4	28	2	56	11	55	7	71	7	103	20
AD-1700813.1	9	1	14	3	14	4	16	4	23	4	25	1	45	2	67	10

Duplex ID	Transfection								Free Uptake							
	10 nM		1 nM		0.1 nM		0.01nM		250 nM		100 nM		10 nM		1 nM	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1700968.1	6	2	10	1	12	3	8	3	10	3	19	3	35	11	41	29
AD-1700780.1	1	0	3	1	9	2	7	2	4	1	4	1	10	4	22	9
AD-1700324.1	8	2	9	2	10	1	12	4	18	1	20	4	33	6	44	11
AD-1700566.1	10	2	17	3	19	1	20	3	35	5	29	5	51	5	90	9
AD-1700354.1	7	1	11	2	16	2	16	1	9	2	12	3	28	4	49	8
AD-1700365.1	20	2	35	4	42	3	44	5	58	10	61	11	81	5	112	12
AD-1700553.1	2	0	5	1	6	1	6	2	3	0	5	2	8	3	30	11

Example 4. *In vivo* screening of dsRNA Duplexes

(1) Single Dose Study (3mg/kg)

Duplexes of interest, identified from the above *in vitro* studies, were evaluated *in vivo*. In particular, at pre-dose day -14 wild-type mice (C57BL/6) were transduced with 2×10^{10} viral particles of an adeno-associated virus 8 (AAV8) vector encoding human CIDEB intravenously via retro-orbital delivery. In particular, mice were administered an AAV8 encoding a portion of human CIDEB mRNA encoding the open reading frame and 3' UTR of human CIDEB mRNA referenced as NM_001393338.1, referred to as VCAV-07736-AAV8.HsCideb-FL-trd.

At day 0, groups of three mice were subcutaneously administered a single 3 mg/kg dose of the duplexes of interest or phosphate-buffered saline (PBS). **Table 11** provides the treatment design and provides the duplexes of interest. On day 7 after dosing, animals were sacrificed, and liver samples were collected and snap-frozen in liquid nitrogen. Liver mRNA was extracted and analyzed by the RT-QPCR method.

For all samples, human CIDEB Cq values were first normalized to Gapdh Cq values as a reference gene to calculate the liver CIDEB mRNA levels for each animal. For each group, the liver CIDEB mRNA levels relative to Gapdh from individual treated animals were normalized to the group mean ($\pm$ standard deviation [SD]) of relative CIDEB mRNA levels from the PBS group.

The data were expressed as percent of baseline value and presented as mean plus standard deviation. The results, listed in **Table 12** and shown in **Figure 1**, demonstrate that the exemplary duplex agents tested effectively reduce the level of the human CIDEB messenger RNA *in vivo*.

Table 11. Study Design for *In Vivo* Single Dose Study

Test Group	Test Material	No. of Females	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)	Route/Regimen
G1	PBS	3	3	10	0.3	SC/Single dose
G2	Naïve	3	3	10	0.3	N/A
G3	AD-1700554.3	3	3	10	0.3	SC/Single dose
G4	AD-1700383.3	3	3	10	0.3	SC/Single dose
G5	AD-1700374.3	3	3	10	0.3	SC/Single dose
G6	AD-1700995.3	3	3	10	0.3	SC/Single dose
G7	AD-1720294.3	3	3	10	0.3	SC/Single dose
G8	AD-1720314.3	3	3	10	0.3	SC/Single dose
G9	AD-1700556.3	3	3	10	0.3	SC/Single dose
G10	AD-1700782.3	3	3	10	0.3	SC/Single dose
G11	AD-1700376.3	3	3	10	0.3	SC/Single dose
G12	AD-1700544.3	3	3	10	0.3	SC/Single dose
G13	AD-1699964.3	3	3	10	0.3	SC/Single dose
G14	AD-1700314.3	3	3	10	0.3	SC/Single dose
G15	AD-1700148.3	3	3	10	0.3	SC/Single dose
G16	AD-1700573.3	3	3	10	0.3	SC/Single dose
G17	AD-1699971.3	3	3	10	0.3	SC/Single dose
G18	AD-1700473.3	3	3	10	0.3	SC/Single dose
G19	AD-1700369.3	3	3	10	0.3	SC/Single dose
G20	AD-1699976.5	3	3	10	0.3	SC/Single dose
G21	AD-1700555.3	3	3	10	0.3	SC/Single dose
G22	AD-1720315.3	3	3	10	0.3	SC/Single dose
G23	AD-1700821.3	3	3	10	0.3	SC/Single dose
G24	AD-1699970.3	3	3	10	0.3	SC/Single dose

Table 12. qPCR Results for *In Vivo* Single Dose Study

Grp	Mouse #	Duplex	# message/mouse				avg/mouse	grp avg	stdev/grp.
1	1	PBS	131.8	124.7	123.0	118.0	124.4	104.3	33.86
	2		123.0	122.1	125.6	123.0	123.4		
	3		65.9	67.3	63.7	64.1	65.2		
2	4	Naïve	65.9	69.2	64.1	59.8	64.7	79.3	20.60
	5								
	6		97.2	95.8	90.6	91.9	93.9		
3	7	AD-1700554.3	31.2	34.1	32.7	32.7	32.7	19.6	12.56
	8		19.2	18.2	18.8	18.2	18.6		
	9		8.0	7.8	7.6	7.1	7.6		
4	10	AD-1700383.3	69.2	65.9	65.9	63.2	66.0	66.5	1.71
	11		68.7	61.9	65.0	64.5	65.0		
	12		70.1	70.1	67.8	65.4	68.4		
5	13	AD-1700374.3	12.7	12.7	13.5	12.1	12.7	17.4	5.40
	14		16.0	15.7	15.9	17.2	16.2		
	15		23.8	23.0	22.8	23.6	23.3		
6	16	AD-1700995.3	26.9	30.5	26.8	28.9	28.3	29.0	4.39
	17		34.1	34.8	32.5	33.4	33.7		
	18		25.3	25.1	24.8	24.8	25.0		
7	19	AD-1720294.3						48.5	35.86
	20		14.4	16.1	13.3	14.5	14.6		
	21		37.8	40.0	36.8	39.5	38.5		
8	22	AD-1720314.3	26.9	30.5	27.1	27.5	28.0	35.6	12.70
	23		47.2	53.2	51.0	49.6	50.2		
	24		27.1	29.9	29.9	26.9	28.5		
9	25	AD-1700556.3	14.2	16.1	16.6	16.5	15.9	17.5	3.58
	26		14.3	14.5	16.6	14.7	15.1		
	27		21.9	21.7	21.3	21.6	21.6		
10	28	AD-1700782.3	21.0	22.7	20.9	22.0	21.6	19.7	4.09
	29		15.0	15.4	15.1	14.7	15.0		
	30		23.1	22.3	22.2	22.3	22.5		

Grp	Mouse #	Duplex	# message/mouse				avg/mouse	grp avg	stdev/grp.
11	31	AD-1700376.3	14.5	14.1	14.4	14.5	14.4	15.7	8.40
	32		8.2	7.8	8.1	8.1	8.1		
	33		23.6	26.4	26.0	22.8	24.7		
12	34	AD-1700544.3	28.5	27.1	27.5	26.9	27.5	22.8	11.68
	35		31.2	32.0	31.6	30.5	31.3		
	36		9.2	10.1	9.7	9.0	9.5		
13	37	AD-1699964.3	12.1	12.4	12.2	12.3	12.3	11.0	1.61
	38		8.4	9.3	9.5	9.4	9.2		
	39		11.6	11.6	11.3	11.1	11.4		
14	40	AD-1700314.3	10.9	11.1	10.9	10.9	11.0	11.3	2.31
	41		13.9	14.0	13.7	13.6	13.8		
	42		9.5	9.5	9.1	8.9	9.2		
15	43	AD-1700148.3	46.0	46.3	47.6	47.9	46.9	44.1	6.55
	44		36.6	36.6	36.3	36.8	36.6		
	45		48.2	48.9	47.9	49.6	48.7		
16	46	AD-1700573.3	20.4	20.7	21.0	21.9	21.0	25.6	4.38
	47		29.7	30.5	29.9	28.9	29.8		
	48		25.5	26.4	25.7	26.0	25.9		
17	49	AD-1699971.3	43.2	44.7	40.8	41.4	42.5	29.3	16.43
	50		11.1	11.0	11.0	10.5	10.9		
	51		35.3	34.8	35.6	32.0	34.4		
18	52	AD-1700473.3	46.6	45.0	46.6	45.6	46.0	23.5	20.90
	53		19.5	20.1	20.1	20.6	20.1		
	54		4.6	4.5	4.6	4.6	4.6		
19	55	AD-1700369.3	13.9	13.2	13.3	13.2	13.4	14.2	0.75
	56		15.1	14.8	15.0	14.7	14.9		
	57		14.1	13.9	14.0	15.1	14.3		
20	58	AD-1699976.5	16.0	15.5	14.4	15.2	15.3	24.1	14.83
	59		41.4	42.9	42.9	37.8	41.3		
	60		15.4	15.5	16.5	16.1	15.9		
21	61	AD-1700555.3	3.7	3.7	3.6	3.3	3.6	9.1	5.50
	62		16.1	15.7	13.0	13.5	14.6		
	63		10.2	9.1	8.8	8.7	9.2		
22	64	AD-1720315.3	45.6	44.1	42.9	45.3	44.5	64.9	23.44
	65		90.0	91.3	91.3	89.4	90.5		
	66		57.4	61.1	59.8	60.6	59.7		
23	67	AD-1700821.3	13.3	13.8	13.9	13.6	13.6	14.8	2.18
	68		17.1	17.3	18.2	16.9	17.4		
	69		13.3	13.9	13.7	13.3	13.5		
24	70		27.1	27.5	24.6	25.1	26.1	19.0	7.21

Grp	Mouse #	Duplex	# message/mouse				avg/mouse	grp avg	stdev/grp.
	71	AD-1699970.3	12.9	11.4	11.8	10.6	11.7		
	72		19.1	19.5	18.5	19.6	19.2		

(2) Multiple Dose Study (0.75 mg/kg and 1.5 mg/kg)

Duplexes of interest, identified from the above *in vitro* studies, were evaluated *in vivo*. In particular, at pre-dose day -14 wild-type mice (C57BL/6) were transduced with 2×10^{10} viral particles of an adeno-associated virus 8 (AAV8) vector encoding human CIDEB by intravenously via retro-orbital delivery. In particular, mice were administered an AAV8 encoding a portion of human CIDEB mRNA encoding the open reading frame and 3' UTR of human CIDEB mRNA referenced as NM_001393338.1, referred to as VCAV-07736-AAV8.HsCideb-FL-trd.

At day 0, groups of three mice were subcutaneously administered a single 0.75 or 1.5 mg/kg dose of the duplexes of interest or phosphate buffered saline (PBS). **Table 13** provides the study design and provides the duplexes of interest. On day 10 after dosing, animals were sacrificed, and liver samples were collected and snap-frozen in liquid nitrogen. Liver mRNA was extracted and analyzed by the RT-QPCR method.

For all samples, human CIDEB Cq values were first normalized to Gapdh Cq values as a reference gene to calculate the liver CIDEB mRNA levels for each animal. For each group, the liver CIDEB mRNA levels relative to Gapdh from individual treated animals were normalized to the group mean ($\pm$ standard deviation [SD]) of relative CIDEB mRNA levels from the PBS group.

The data were expressed as percent of baseline value and presented as mean plus standard deviation. The results, listed in **Table 14** and shown in **Figure 2**, demonstrate that the exemplary duplex agents tested effectively dose-dependently reduced the level of the human CIDEB messenger RNA *in vivo*.

Table 13. Study Design for *In Vivo* Multi-Dose Study

Test Group	Test Material	No. of Females	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)	Route/Regimen
G1	PBS	3	3	10	0.3	SC/Single dose
G2	Naïve	3	3	10	0.3	N/A
G3	AD-1700555.3	3	1.5	10	0.15	SC/Single dose
G4	AD-1700555.3	3	0.75	10	0.075	SC/Single dose
G5	AD-1699964.3	3	1.5	10	0.15	SC/Single dose
G6	AD-1699964.3	3	0.75	10	0.075	SC/Single dose
G7	AD-1700314.3	3	1.5	10	0.15	SC/Single dose

Test Group	Test Material	No. of Females	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)	Route/ Regimen
G8	AD-1700314.3	3	0.75	10	0.075	SC/Single dose
G9	AD-1700369.3	3	1.5	10	0.15	SC/Single dose
G10	AD-1700369.3	3	0.75	10	0.075	SC/Single dose
G11	AD-1700821.3	3	1.5	10	0.15	SC/Single dose
G12	AD-1700821.3	3	0.75	10	0.075	SC/Single dose
G13	AD-1700376.3	3	1.5	10	0.15	SC/Single dose
G14	AD-1700376.3	3	0.75	10	0.075	SC/Single dose
G15	AD-1700374.3	3	1.5	10	0.15	SC/Single dose
G16	AD-1700374.3	3	0.75	10	0.075	SC/Single dose
G17	AD-1700556.3	3	1.5	10	0.15	SC/Single dose
G18	AD-1700556.3	3	0.75	10	0.075	SC/Single dose
G19	AD-1699976.5	3	1.5	10	0.15	SC/Single dose
G20	AD-1699976.5	3	0.75	10	0.075	SC/Single dose

Table 14. qPCR Results for *In Vivo* Multi-Dose Study

Grp	Mouse #	Duplex	# message/mouse				avg/mouse	grp avg	stdev/grp.
1	1	PBS	101.0	102.4	101.7	99.6	101.2	100.0	1.01
	2		103.1	101.0	97.5	96.9	99.6		
	3		100.3	101.0	97.5	98.2	99.3		
2	4	Naïve	68.5	71.9	66.2	70.9	69.4	99.7	69.67
	5		177.1	191.1	175.8	173.4	179.3		
	6		50.1	51.9	50.5	48.4	50.2		
3	7	AD-1700555.3 1.5mpk	10.0	10.9	10.9	10.0	10.5	20.8	11.21
	8		18.9	20.4	18.5	18.9	19.1		
	9		32.2	34.0	33.1	31.5	32.7		
4	10	AD-1700555.3 0.75mpk	43.7	46.8	45.5	42.8	44.7	42.2	2.16
	11		41.0	43.4	40.2	40.4	41.2		
	12		41.3	42.5	40.4	38.5	40.7		

Grp	Mouse #	Duplex	# message/mouse				avg/mouse	grp avg	stdev/grp.
5	13	AD-1699964.3 1.5mpk	37.7	39.3	37.0	36.7	37.7	26.3	16.14
	14								
	15		15.0	15.0	14.8	14.6	14.9		
6	16	AD-1699964.3 0.75mpk	67.6	67.1	64.8	67.1	66.6	69.6	13.85
	17		84.9	87.3	83.8	82.6	84.6		
	18		59.2	57.6	55.6	57.2	57.4		
7	19	AD-1700314.3 1.5mpk	22.1	22.1	21.8	21.8	22.0	25.2	0.66
	20		25.1	26.9	26.1	24.6	25.7		
	21		24.2	25.4	24.6	24.7	24.7		
8	22	AD-1700314.3 0.75mpk						60.9	7.08
	23		57.2	56.4	54.5	55.6	55.9		
	24		67.1	67.6	63.9	65.3	66.0		
9	25	AD-1700369.3 1.5mpk	30.2	30.4	30.0	30.0	30.2	34.6	8.85
	26		43.7	45.2	44.9	45.5	44.8		
	27		28.2	28.2	29.4	29.6	28.9		
10	28	AD-1700369.3 0.75mpk	47.8	49.1	50.8	50.5	49.6	48.3	38.49
	29		9.1	9.4	9.1	9.2	9.2		
	30		86.7	89.1	87.3	81.5	86.2		
11	31	AD-1700821.3 1.5mpk	37.0	37.5	37.2	37.7	37.4	25.0	21.40
	32		0.3	0.2	0.2	0.3	0.2		
	33		37.5	37.2	37.7	36.7	37.3		
12	34	AD-1700821.3 0.75mpk	51.9	54.5	52.3	52.6	52.8	43.5	16.48
	35		24.1	24.6	24.6	24.6	24.4		
	36		53.0	54.5	54.5	50.5	53.1		
13	37	AD-1700376.3 1.5mpk	29.6	30.9	28.8	28.4	29.4	37.1	6.74
	38		38.5	43.4	39.3	38.3	39.9		
	39		43.1	41.9	40.7	42.5	42.0		
14	40	AD-1700376.3 0.75mpk	79.8	80.9	79.8	80.9	80.3	68.3	23.43
	41		42.5	42.2	41.6	38.8	41.3		
	42		82.6	84.3	83.8	82.0	83.2		
15	43	AD-1700374.3 1.5mpk	22.0	22.9	22.6	22.8	22.6	32.2	12.26
	44		28.2	27.1	28.8	28.2	28.1		
	45		47.4	47.8	44.0	44.9	46.0		
16	46	AD-1700374.3 0.75mpk	72.9	76.5	75.5	76.5	75.4	56.7	25.04
	47		29.2	28.0	27.4	28.2	28.2		
	48		68.5	66.6	64.8	65.7	66.4		
17	49	AD-1700556.3 1.5mpk	63.0	64.8	58.0	60.9	61.7	29.6	30.64
	50		27.6	26.5	26.9	25.2	26.6		
	51		0.7	0.6	0.6	0.6	0.6		
18	52		63.9	64.4	65.3	62.2	63.9	89.3	22.06

Grp	Mouse #	Duplex	# message/mouse				avg/mouse	grp avg	stdev/grp.
	53	AD-1700556.3 0.75mpk	98.9	102.4	102.4	96.9	100.1		
	54		105.3	101.7	103.8	104.5	103.8		
19	55	AD-1699976.5 1.5mpk	35.7	36.0	37.7	38.3	36.9	35.3	4.49
	56		30.0	29.8	31.3	30.0	30.3		
	57		37.5	40.7	40.4	36.7	38.8		
20	58	AD-1699976.5 0.75mpk	62.2	61.3	61.7	59.6	61.2	72.1	18.75
	59		97.5	95.5	93.6	88.5	93.8		
	60		62.2	61.3	61.3	60.9	61.4		

CIDEB Sequences

SEQ ID NO: 1

5 >NM_001393338.1 Homo sapiens cell death inducing DFFA like effector b
(CIDEB), transcript variant 7, mRNA
CCCTTCCGGTGGAGCCAGCGCTGCGACCGCCTGCAGAAGGTTGACTGCGTGGTAGGGGGCCAGAGCAAGCCGAAGG
CAAGCACGATGGCGCTCACCAGCCGGCCCCACCCGCGCCCCGTGCCGCCCGGAGCCCCAGCGGGCGCCCCGAGCCGT
10 GCCAGCGTCACGCTGTAGCAGCCGAGCATCAGCCCCGAAAGGAAGCACGAAAGCGGTTCAGAGTCTCCAGGCTCAGGTG
GGCGGGCGCGTGGACCGGCGACGGGTGGCACAGCTGGCATAACGCGGTCCCTCCACAGGTGGCGGTAGACGGCGGGCCG
GGACGGCGAGCAACAGGGCGGCCAGCCAGACCGCCAGCAGCAGGCGGGCGGGCCAGGGCCGGGTGCGCAGCCGAGGC
GCCAGGAAGGGCGGGTGTACTGCGAGGCAGCGCTGCAGGCTGAGCAGGCCGGTGAGCAGCACGCTGGCGTACATGCT
GAGCGCGCACACGTAGTACACCGCCTTGACAGCCCGCTGGCCAGCGGCCAGGCCTGCCGGGTGAGGAAGGCCACAA
15 AGAGCGGCGTGAGCAGCAGCACCGCGCCGTGGCCAGCGCCAGGTGCAGCACAAAGCGTGGCCGCCAGCGGTGCCCC
CGTGCAGGCCGCCAGCCCGCAAGCTCCACACCACGAAGCCGTTGCCAGGCAGCCCCAGCAGCGCCGCCAGCAGCAG
GAAGGCTGTGCTGTGGCCCGGAAGTCTTCCAGCTCAGCAGTGTCTCGTTCCCTGGGGGACGGTAGCAGACCGACA
TCCTTCTGGGCTACAGCCTGCCTCTTTTCTGCCTGGGAGTCTGACTTCCACGAGGACCCAGACCCACCTCAAAC
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40 TCAAATATTTACCACTAA

SEQ ID NO:2

>Reverse complement of SEQ ID NO: 1

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CTGGCTCCACCGGAAGGG

We Claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO: 1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO: 2.

2. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell, wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein said antisense strand comprises a region of complementarity to an mRNA encoding CIDEb which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense sequences listed in Tables 3-6.

3. The dsRNA agent of claim 1 or 2, wherein

(a) the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700555 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700555;

(b) the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700821 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700821;

(c) the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700369 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700369;

(d) the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1699976 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1699976;

(e) the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700374 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700374;

(f) the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700314 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700314;

(g) the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700376 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700376;

(h) the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1699964 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1699964; or

(i) the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1700556 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1700556.

4. The dsRNA agent of any one of claims 1-3, wherein said dsRNA agent comprises at least one modified nucleotide.

5. The dsRNA agent of any one of claims 1-4, wherein substantially all of the nucleotides of the sense strand comprise a modification.

6. The dsRNA agent of any one of claims 1-4, wherein substantially all of the nucleotides of the antisense strand comprise a modification.

7. The dsRNA agent of any one of claims 1-4, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification.

8. A double stranded RNA (dsRNA) agent for inhibiting expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell, wherein the double stranded RNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO: 1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO: 2,

wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and

wherein the sense strand is conjugated to a ligand attached at the 3'-terminus.

9. The dsRNA agent of claim 8, wherein all of the nucleotides of the sense strand comprise a modification.

10. The dsRNA agent of claim 8, wherein all of the nucleotides of the antisense strand comprise a modification.

11. The dsRNA agent of claim 8, wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

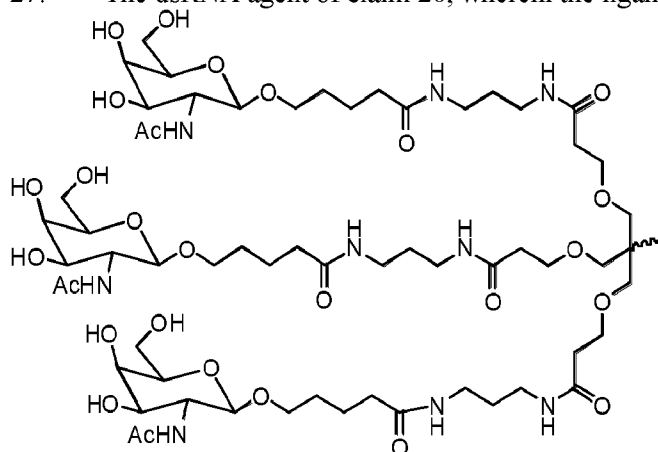
12. The dsRNA agent of any one of claims 4-11, wherein at least one of said modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a glycol modified nucleotide, and a 2-O-(N-methylacetamide) modified nucleotide, and combinations thereof.

13. The dsRNA agent of claim 12, wherein the nucleotide modifications are 2'-O-methyl and/or 2'-fluoro modifications.
14. The dsRNA agent of any one of claims 1-13, wherein the region of complementarity is at least 17 nucleotides in length.
15. The dsRNA agent of any one of claims 1-14, wherein the region of complementarity is 19 to 30 nucleotides in length.
16. The dsRNA agent of claim 15, wherein the region of complementarity is 19-25 nucleotides in length.
17. The dsRNA agent of claim 16, wherein the region of complementarity is 21 to 23 nucleotides in length.
18. The dsRNA agent of any one of claims 1-17, wherein each strand is no more than 30 nucleotides in length.
19. The dsRNA agent of any one of claims 1-18, wherein each strand is independently 19-30 nucleotides in length.
20. The dsRNA agent of claim 19, wherein each strand is independently 19-25 nucleotides in length.
21. The dsRNA agent of claim 19, wherein each strand is independently 21-23 nucleotides in length.
22. The dsRNA agent of any one of claims 1-21, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.
23. The dsRNA agent of any one of claim 22, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.
24. The dsRNA agent of any one of claims 1-7 and 12-23 further comprising a ligand.

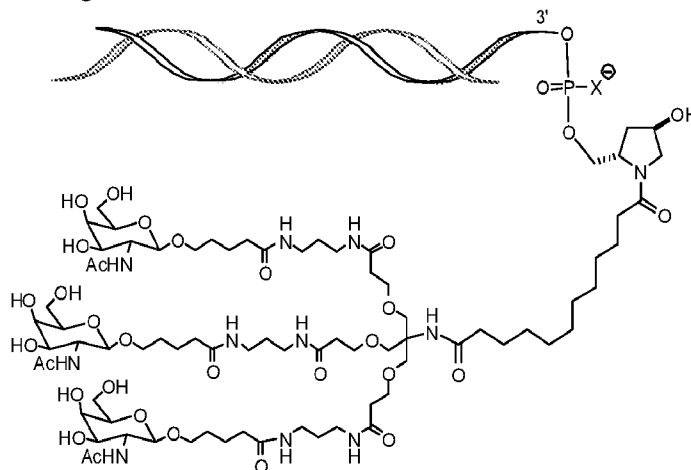
25. The dsRNA agent of claim 24, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

26. The dsRNA agent of claim 8 or 25, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

27. The dsRNA agent of claim 26, wherein the ligand is



28. The dsRNA agent of claim 27, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic

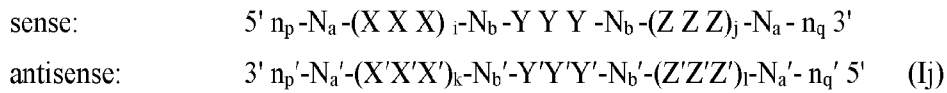


and, wherein X is O or S.

29. The dsRNA agent of claim 28, wherein the X is O.

30. The dsRNA agent of claim 2, wherein the region of complementarity comprises any one of the antisense sequences in Tables 3-6.

31. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell, wherein said dsRNA agent comprises a sense strand complementary to an antisense strand, wherein said antisense strand comprises a region complementary to part of an mRNA encoding CIDEb, wherein each strand is about 14 to about 30 nucleotides in length, wherein said dsRNA agent is represented by formula (Ij):



wherein:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

each n_p , n_p' , n_q , and n_q' , each of which may or may not be present, independently represents an overhang nucleotide;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

wherein the sense strand is conjugated to at least one ligand.

32. The dsRNA agent of claim 31, wherein i is 0; j is 0; i is 1; j is 1; both i and j are 0; or both i and j are 1.

33. The dsRNA agent of claim 31, wherein k is 0; l is 0; k is 1; l is 1; both k and l are 0; or both k and l are 1.

34. The dsRNA agent of claim 31, wherein XXX is complementary to X'X'X', YYY is complementary to Y'Y'Y', and ZZZ is complementary to Z'Z'Z'.

35. The dsRNA agent of claim 31, wherein the YYY motif occurs at or near the cleavage site of the sense strand.

36. The dsRNA agent of claim 31, wherein the Y'Y'Y' motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end.

37. The dsRNA agent of claim 31, wherein formula (Ij) is represented by formula (Ik):

sense: $5' n_p - N_a - Y Y Y - N_a - n_q 3'$

antisense: $3' n_p - N_a - Y'Y'Y' - N_a - n_q 5'$ (Ik).

38. The dsRNA agent of claim 31, wherein formula (Ij) is represented by formula (Il):

sense: $5' n_p - N_a - Y Y Y - N_b - Z Z Z - N_a - n_q 3'$

antisense: $3' n_p - N_a - Y'Y'Y' - N_b - Z'Z'Z' - N_a - n_q 5'$ (Il)

wherein each N_b and N_b' independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides.

39. The dsRNA agent of claim 31, wherein formula (Ij) is represented by formula (Im):

sense: $5' n_p - N_a - X X X - N_b - Y Y Y - N_a - n_q 3'$

antisense: $3' n_p - N_a - X'X'X' - N_b - Y'Y'Y' - N_a - n_q 5'$ (Im)

wherein each N_b and N_b' independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides.

40. The dsRNA agent of claim 31, wherein formula (Ij) is represented by formula (In):

sense: $5' n_p - N_a - X X X - N_b - Y Y Y - N_b - Z Z Z - N_a - n_q 3'$

antisense: $3' n_p - N_a - X'X'X' - N_b - Y'Y'Y' - N_b - Z'Z'Z' - N_a - n_q 5'$ (In)

wherein each N_b and N_b' independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides and each N_a and N_a' independently represents an oligonucleotide sequence comprising 2-10 modified nucleotides.

41. The dsRNA agent of any one of claims 31-40, wherein the region of complementarity is at least 17 nucleotides in length.

42. The dsRNA agent of any one of claims 31-40, wherein the region of complementarity is 19 to 30 nucleotides in length.

43. The dsRNA agent of claim 42, wherein the region of complementarity is 19-25 nucleotides in length.

44. The dsRNA agent of claim 43, wherein the region of complementarity is 21 to 23 nucleotides in length.

45. The dsRNA agent of any one of claims 31-44, wherein each strand is no more than 30 nucleotides in length.

46. The dsRNA agent of any one of claims 31-44, wherein each strand is independently 19-30 nucleotides in length.

47. The dsRNA agent of any one of claims 31-46, wherein the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-alkyl, 2'-O-allyl, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-deoxy, 2'-hydroxyl, and combinations thereof.

48. The dsRNA agent of claim 47, wherein the modifications on the nucleotides are 2'-O-methyl and/or 2'-fluoro modifications.

49. The dsRNA agent of claim any one of claims 31-47, wherein the Y' is a 2'-O-methyl or 2'-fluoro modified nucleotide.

50. The dsRNA agent of any one of claims 31-49, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

51. The dsRNA agent of any one of claims 31-50, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

52. The dsRNA agent of any one of claims 31-51, wherein the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

53. The dsRNA agent of claim 52, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand.

54. The dsRNA agent of claim 53, wherein said strand is the antisense strand.

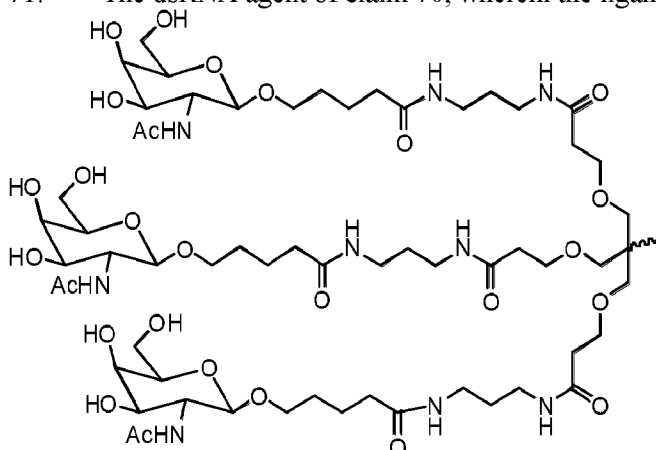
55. The dsRNA agent of claim 53, wherein said strand is the sense strand.

56. The dsRNA agent of claim 52, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.
57. The dsRNA agent of claim 56, wherein said strand is the antisense strand.
58. The dsRNA agent of claim 56, wherein said strand is the sense strand.
59. The dsRNA agent of claim 52, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at both the 5'- and 3'-terminus of one strand.
60. The dsRNA agent of claim 31, wherein the base pair at the 1 position of the 5'-end of the antisense strand of the duplex is an AU base pair.
61. The dsRNA agent of claim 31, wherein $p' > 0$.
62. The dsRNA agent of claim 31, wherein $p' = 2$.
63. The dsRNA agent of claim 62, wherein $q' = 0$, $p = 0$, $q = 0$, and p' overhang nucleotides are complementary to the target mRNA.
64. The dsRNA agent of claim 62, wherein $q' = 0$, $p = 0$, $q = 0$, and p' overhang nucleotides are non-complementary to the target mRNA.
65. The dsRNA agent of claim 31, wherein the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.
66. The dsRNA agent of claim 31, wherein at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage.
67. The dsRNA agent of claim 66, wherein all n_p' are linked to neighboring nucleotides via phosphorothioate linkages.
68. The dsRNA agent of claim 31, wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

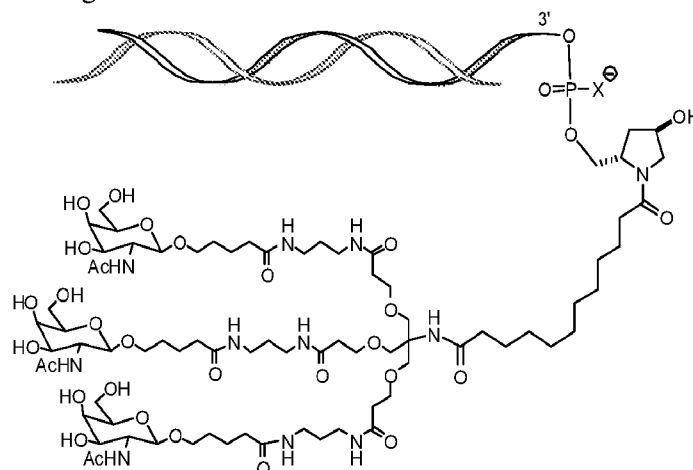
69. The dsRNA agent of any one of claims 31-68, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

70. The dsRNA agent of claim 69, wherein the ligand is one or more N-acetylgalactosamine (GalNAc) derivatives attached through a monovalent, bivalent, or trivalent branched linker.

71. The dsRNA agent of claim 70, wherein the ligand is



72. The dsRNA agent of claim 71, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic

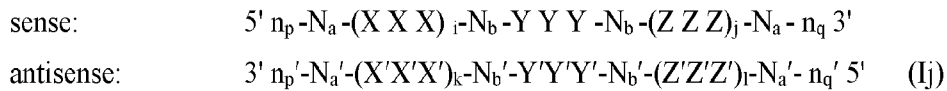


and, wherein X is O or S.

73. The dsRNA agent of claim 72, wherein the X is O.

74. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell, wherein the dsRNA agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CIDEb, wherein each strand is about 14 to about 30 nucleotides in length, wherein the

dsRNA agent is represented by formula (Ij):



wherein:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

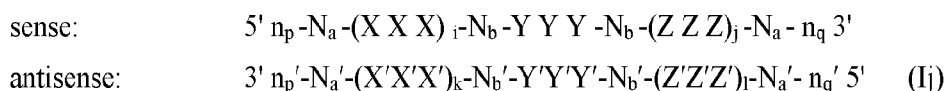
each n_p , n_p' , n_q , and n_q' , each of which may or may not be present independently represents an overhang nucleotide;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

wherein the sense strand is conjugated to at least one ligand.

75. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell, wherein the dsRNA agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CIDEb, wherein each strand is about 14 to about 30 nucleotides in length, wherein the dsRNA agent is represented by formula (Ij):



wherein:

i, j, k, and l are each independently 0 or 1;

each n_p , n_q , and n_q' , each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q' are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

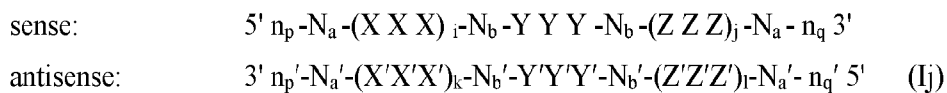
each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

wherein the sense strand is conjugated to at least one ligand.

76. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell, wherein the dsRNA agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CIDEb, wherein each strand is about 14 to about 30 nucleotides in length, wherein the dsRNA agent is represented by formula (Ij):



wherein:

i, j, k, and l are each independently 0 or 1;

each n_p , n_q , and n_q' , each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q' are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

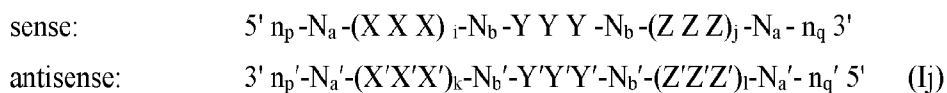
each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

77. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell, wherein the dsRNA agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CIDEb, wherein each strand is about 14 to about 30 nucleotides in length, wherein the dsRNA agent is represented by formula (Ij):



wherein:

i, j, k, and l are each independently 0 or 1;

each n_p , n_q , and n_q' , each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q' are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

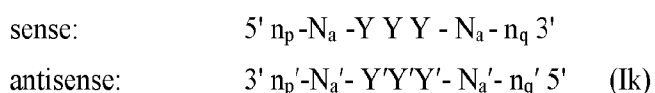
XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y';

wherein the sense strand comprises at least one phosphorothioate linkage; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

78. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell, wherein the dsRNA agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CIDEb, wherein each strand is about 14 to about 30 nucleotides in length, wherein the dsRNA agent is represented by formula (Ik):



wherein:

each n_p , n_q , and $n_{q'}$, each of which may or may not be present, independently represents an overhang nucleotide;

p , q , and q' are each independently 0-6;

$n_p > 0$ and at least one $n_{p'}$ is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

YYY and Y'Y'Y' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-fluoro modifications;

wherein the sense strand comprises at least one phosphorothioate linkage; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

79. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO: 1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO: 2,

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

wherein the sense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus,

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

wherein the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and

wherein the sense strand is conjugated to one or more GalNAc derivatives attached through a monovalent, bivalent or trivalent branched linker at the 3'-terminus.

80. The dsRNA agent of claim 79, wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides.

81. The dsRNA agent of any one of claims 3, 31, and 74-80 wherein the region of complementarity comprises any one of the antisense sequences listed in Tables 3-6.

82. The dsRNA agent of any one of claims 1-81, wherein the sense strand and the antisense strand comprise nucleotide sequences selected from the group consisting of the nucleotide sequences of any one of the agents listed in Tables 3-6.

83. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of cell death-inducing DFFA-like effector b (CIDEb) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises a nucleotide sequence of any one of the agents in Tables 3-6, and the antisense strand comprises a nucleotide sequence of any one of the agents in in Tables 3-6,

wherein substantially all of the nucleotide of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and

wherein the dsRNA agent is conjugated to a ligand.

84. The dsRNA of any one of claims 1-83 wherein the dsRNA agent targets a hotspot region of an mRNA encoding CIDEb.

85. A dsRNA agent that targets a hotspot region of a cell death-inducing DFFA-like effector b (CIDEb) mRNA.

86. A cell containing the dsRNA agent of any one of claims 1-85.

87. A vector encoding at least one strand of the dsRNA agent of any one of claims 1-85.

88. A pharmaceutical composition for inhibiting expression of the cell death-inducing DFFA-like effector b (CIDEb) gene comprising the dsRNA agent of any one of claims 1-85.

89. The pharmaceutical composition of claim 88, wherein the agent is formulated in an unbuffered solution.

90. The pharmaceutical composition of claim 89, wherein the unbuffered solution is saline or water.

91. The pharmaceutical composition of claim 88, wherein the agent is formulated with a buffered solution.

92. The pharmaceutical composition of claim 91, wherein said buffered solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

93. The pharmaceutical composition of claim 91, wherein the buffered solution is phosphate buffered saline (PBS).

94. A method of inhibiting cell death-inducing DFFA-like effector b (CIDEB) expression in a cell, the method comprising contacting the cell with the agent of any one of claims 1-84, or a pharmaceutical composition of any one of claims 88-93, thereby inhibiting expression of CIDEB in the cell.

95. The method of claim 94, wherein said cell is within a subject.

96. The method of claim 95, wherein the subject is a human.

97. The method of any one of claims 94-96, wherein the CIDEB expression is inhibited by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or to below the level of detection of CIDEB expression.

98. The method of claim 97, wherein the human subject suffers from an CIDEB-associated disease, disorder, or condition.

99. The method of claim 98, wherein the CIDEB-associated disease, disorder, or condition is a chronic inflammatory disease.

100. The method of claim 99, wherein the chronic inflammatory disease is chronic inflammatory liver disease.

101. The method of claim 100, wherein the chronic inflammatory liver disease is associated with the accumulation and/or expansion of lipid droplets in the liver.

102. The method of claim 100, wherein the chronic inflammatory liver disease is selected from the group consisting of accumulation of fat in the liver, inflammation of the liver, liver fibrosis, fatty liver disease (steatosis), nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD) and cirrhosis of the liver.

103. The method of claim 102, wherein the chronic inflammatory liver disease is nonalcoholic steatohepatitis (NASH).

104. A method of inhibiting the expression of CIDEB in a subject, the method comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-85, or a pharmaceutical composition of any one of claims 88-93, thereby inhibiting the expression of CIDEB in said subject.

105. A method of treating a subject suffering from a CIDEB-associated disease, disorder, or condition, comprising administering to the subject a therapeutically effective amount of the agent of any one of claims 1-85, or a pharmaceutical composition of any one of claims 88-93, thereby treating the subject suffering from a CIDEB-associated disease, disorder, or condition.

106. A method of preventing at least one symptom in a subject having a disease, disorder or condition that would benefit from reduction in expression of a CIDEB gene, comprising administering to the subject a prophylactically effective amount of the agent of any one of claims 1-85, or a pharmaceutical composition of any one of claims 88-93, thereby preventing at least one symptom in a subject having a disease, disorder or condition that would benefit from reduction in expression of a CIDEB gene.

107. A method of reducing the risk of developing chronic liver disease in a subject having nonalcoholic steatohepatitis (NASH), the method comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-85, or a pharmaceutical composition of any one of claims 88-93, thereby reducing the risk of developing chronic liver disease in the subject having NASH.

108. The method of any one of claims 104-107, wherein the CIDEB-associated disease, disorder, or condition is a chronic inflammatory disease.

109. The method of claim 108, wherein the chronic inflammatory disease is chronic inflammatory liver disease.

110. The method of claim 109, wherein the chronic inflammatory liver disease is associated with the accumulation and/or expansion of lipid droplets in the liver.

111. The method of claim 109, wherein the chronic inflammatory liver disease is selected from the group consisting of accumulation of fat in the liver, inflammation of the liver, liver fibrosis, fatty liver disease (steatosis), nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD) and cirrhosis of the liver.

112. The method of claim 111, wherein the chronic inflammatory liver disease is nonalcoholic steatohepatitis (NASH).

113. The method of any one of claims 95-112, wherein the subject is obese.

114. The method of any one of claims 95-113, further comprising administering an additional therapeutic to the subject.

115. The method of any one of claims 95-114, wherein the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg.

116. The method of any one of claims 95-115, wherein the agent is administered to the subject intravenously, intramuscularly, or subcutaneously.

117. The method of any one of claims 95-116, further comprising determining, the level of CIDEA in the subject.

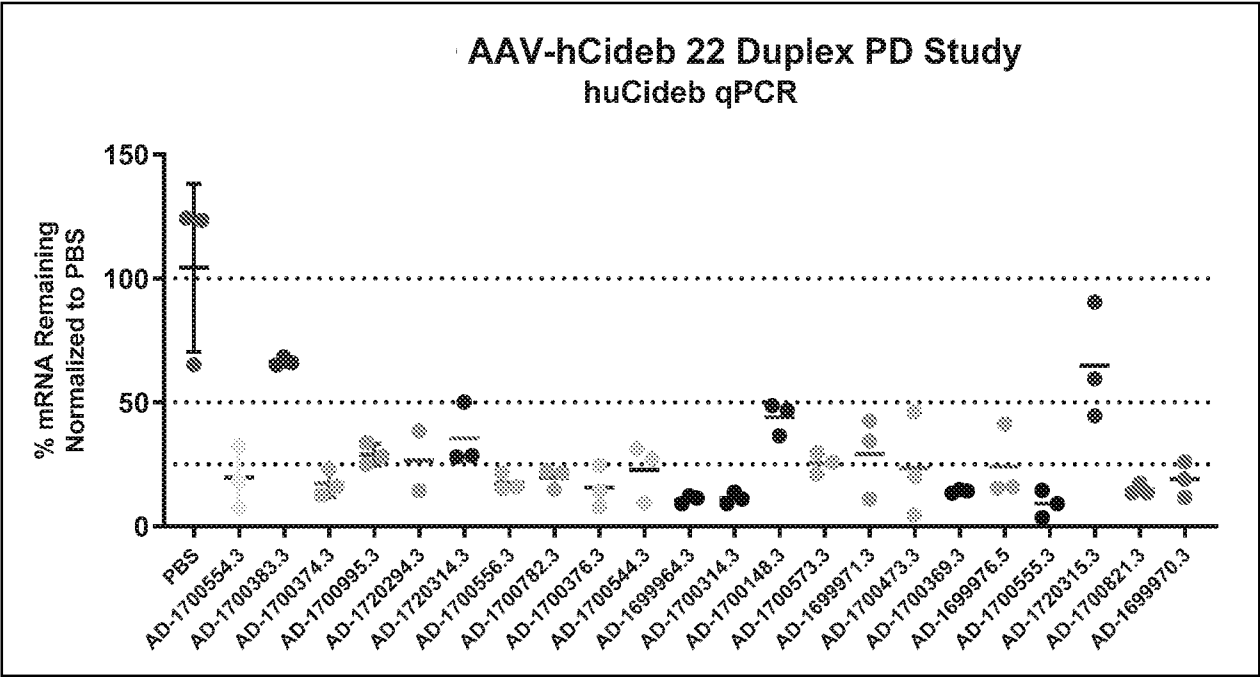


FIG. 1

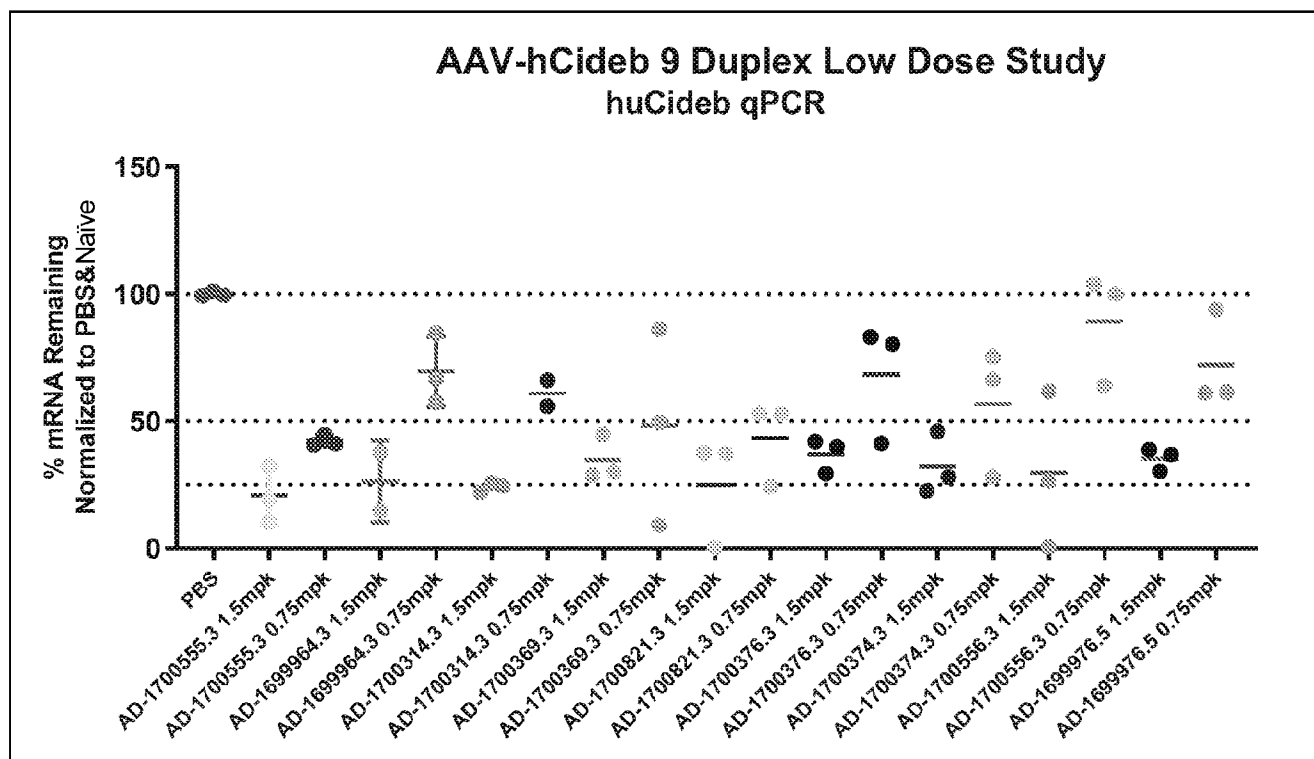


FIG. 2