# 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 incoroporated by reference in its entirety. The XML copy, created on June 14, 2023, is named A108868_1300WO_SL.xml and is 18,162,483 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.

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, 1009-1031, 1018-1040, 1027-1049, 1036-1058, 1045-1067, 1054-1076, 1080-1102, 1089-1111, 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, 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, 1432-1454, 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, 1716-1738, 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, 1838-1860, 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, 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, 1283-1305, 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, 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, 1657-1679, 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, 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, 2102-2124, 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, 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 some embodiments, the 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, 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, 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, 1432-1454, 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, 1716-1738, 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, 1838-1860, 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, 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, 1283-1305, 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, 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, 1657-1679, 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, 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, 2102-2124, 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, 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.

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.

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 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 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 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 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.

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.

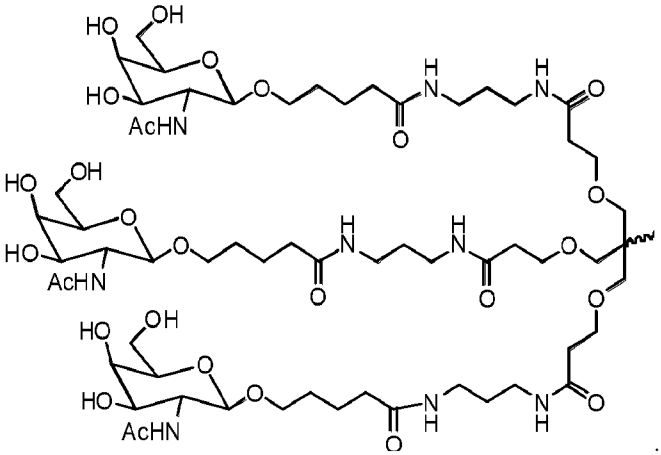
The dsRNA may include at least one strand that comprises a 3' overhang of at least 1 nucleotide; or at least one strand that comprises a 3' overhang of at least 2 nucleotides.

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

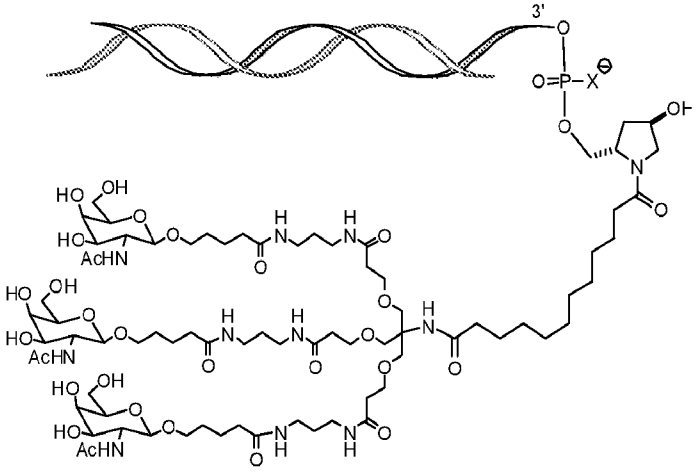
In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

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):

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;

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):

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).

In another embodiment, 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.

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'-flouro 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.

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.

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

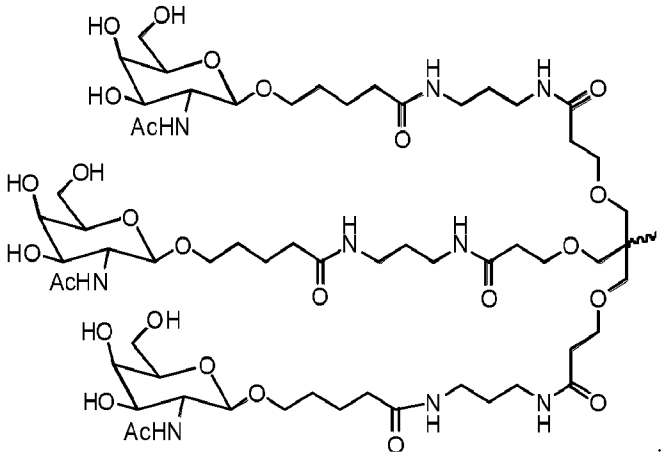
In one embodiment, at least one $n_p'$ is linked to a neighboring nucleotide via a phosphorothioate linkage. In another embodiment, wherein all $n_p'$ are linked to neighboring nucleotides via phosphorothioate linkages.

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

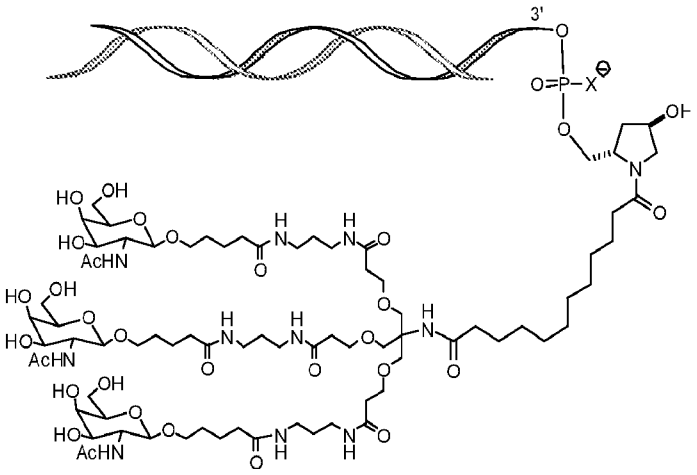
In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

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




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):

sense: $5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (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):

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 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):

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 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.

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):

sense: $5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (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 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.

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):

sense: $5'\ n_p\text{-}N_a\text{-}Y\ Y\ Y\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}Y'Y'Y'\text{-}N_a'\text{-}n_q'\ 5'$ (Ik)

wherein:

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

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.

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.

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 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.

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 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, 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 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 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.

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.

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.

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 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.

## 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 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.*, 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 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 ±10%. In certain embodiments, about means ±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 "CIDEB," 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 CIDEB 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 CIDEB that maintain at least one *in vivo* or *in vitro* activity of a native CIDEB.

CIDEB, 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). CIDEB is an endoplasmic reticulum (ER)- and lipid droplet (LD)-associated protein. As a CIDE family member, 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. For instance, CIDEB mediates VLDL lipidation and maturation by interacting with ApoB; CIDEB is also required for the biogenesis of VLDL transport vesicles and for chylomicron lipidation in the small intestine. CIDEB 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, CIDEB may represent a new therapeutic target for the treatment of obesity, diabetes, and liver steatosis (Li *et al., Diabetes*. 56(10):2523-32. 2007). CIDEB is transcriptionally regulated by hepatocyte nuclear factor 4α (HNF4α), the most abundant transcription factor in the liver; HNF4α is crucial for VLDL-mediated lipid transport and participates in HCV assembly/release. As a key transcriptional coactivator of HNF4α, peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) also regulates HCV production and PGC-1α stimulates VLDL assembly in a CIDEB-dependent manner (Cai *et al.*, *Scientific Reports*, volume 6, Article number: 27778, 2016).

CIDEB is required for HCV entry into hepatocytes and HCV assembly (Xu *et al.*, *J Virol.*, 88, 8433–8444, 2014). CIDEB 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. 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.

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 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.

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 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 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 Hoogstein 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%,



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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 "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, 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 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 CIDEB expression is not 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 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.

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 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 iRNA 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, un-conjugated, 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 lenth 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.

Amenibility 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. 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 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 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, 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 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 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**

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 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.*, 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.*, 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 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).

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-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 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:

$$5'\text{-}\ N_1\text{-}\ldots\ \text{-}N_{n-2}N_{n-1}N_nL96\ 3'$$

may be replaced with

$$5'\text{-}\ N_1\text{-}\ldots\ \text{-}N_{n-2}\underline{s}N_{n-1}\underline{s}N_n\ 3'.$$

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

csusgcagAfaGfGfUfugacugc**guuL96** (SEQ ID NO: 277)

may be replaced with

csusgcagAfaGfGfUfugacugc**gsusu** (SEQ ID NO: 2922)

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 (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. 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



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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.

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 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 in the agent as counterions for substantially all of the phosphodiester and/or phosphorothiotate 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 phosphorothiotate groups present in 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.

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 $CH_2$ 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 --$CH_2$--NH--$CH_2$-, --$CH_2$--N($CH_3$)--O--$CH_2$-- [known as a methylene (methylimino) or MMI backbone], --$CH_2$--O--N($CH_3$)--$CH_2$--, --$CH_2$--N($CH_3$)--N($CH_3$)--$CH_2$-- and --N($CH_3$)--$CH_2$--$CH_2$--[wherein the native phosphodiester backbone is represented as --O--P--O--$CH_2$--] 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 $C_1$ to $C_{10}$ alkyl or $C_2$ to $C_{10}$ alkenyl and alkynyl. Exemplary suitable modifications include $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: $C_1$ to $C_{10}$ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, $SCH_3$, OCN, Cl, Br, CN, $CF_3$, $OCF_3$, $SOCH_3$, $SO_2CH_3$, $ONO_2$, $NO_2$, $N_3$, $NH_2$, 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--$CH_2CH_2OCH_3$, 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'-dimethylaminooxyethoxy, *i.e.*, a $O(CH_2)_2ON(CH_3)_2$ 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--$CH_2$--O--$CH_2$--$N(CH_2)_2$. 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).

Other modifications include 2'-methoxy (2'-$OCH_3$), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) 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 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.

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 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 anal 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 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, these disclosed by Englisch *et al.*, (1991) *Angewandte Chemie, International Edition*, 30:613, and those disclosed by Sanghvi, Y 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 0-6 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)-0-2' 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 cylcohexenyl 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'-0-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 no 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 $GalNAc_3$).

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 1$^{st}$ nucleotide from the 5'-end of the antisense strand, or, the count starting from the 1$^{st}$ 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 than 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 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 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 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' 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 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



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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.

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 "…$N_a$YYY$N_b$…," 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 "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 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 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 6-8phosphorothioate 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 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 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 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):

$$5' \; n_p\text{-}N_a\text{-}(X\,X\,X)_i\text{-}N_b\text{-}Y\,Y\,Y\text{-}N_b\text{-}(Z\,Z\,Z)_j\text{-}N_a\text{-}n_q \; 3' \qquad (I)$$

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;

each $n_p$ and $n_q$ independently represent an overhang nucleotide;

wherein Nb 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.

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.

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).

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 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.

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 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):

5' $n_{q'}$-$N_a'$-(Z'Z'Z')$_k$-$N_b'$-Y'Y'Y'-$N_b'$-(X'X'X')$_l$-$N'_a$-$n_{p'}$ 3' (Ie)

wherein:

k and l 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;

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.

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 17-23nucleotidein 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.

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:

5' $n_{q'}$-$N_a'$-Z'Z'Z'-$N_b'$-Y'Y'Y'-$N_a'$-$n_{p'}$ 3' (Ig);

5' $n_{q'}$-$N_a'$-Y'Y'Y'-$N_b'$-X'X'X'-$n_{p'}$ 3' (Ih); or

5' $n_{q'}$-$N_a'$- Z'Z'Z'-$N_b'$-Y'Y'Y'-$N_b'$- X'X'X'-$N_a'$-$n_{p'}$ 3' (Ii).

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.

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.

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: 5' $n_{p'}$-$N_{a'}$-Y'Y'Y'- $N_{a'}$-$n_{q'}$ 3' (If).

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):

sense: 5' np -Na-(X X X)i -Nb- Y Y Y -Nb -(Z Z Z)j-Na-nq 3'

antisense: 3' np'-Na'-(X'X'X')k-Nb'-Y'Y'Y'-Nb'-(Z'Z'Z')l-Na'-nq' 5'

(Ij)

wherein:

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

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

each Na and Na' 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

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.

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'

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

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)

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 (Il), 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.

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 0modified 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), (Il), (Im), and (In) may be the same or different from each other.

When the RNAi agent is represented by formula (Ij), (Ik), (Il), (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 (Il) 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 a via 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.

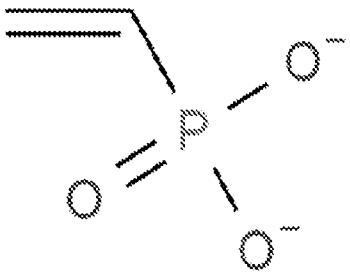
In one embodiment, two RNAi agents represented by formula (Ij), (Ik), (Il), (Im), and (In) are linked to each other at the 5' end, and one or both of the 3' ends and are optionally conjugated to a ligand. Each of the agents can target the same gene or two different genes; or each of the agents can target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

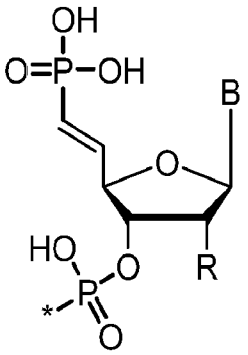
In other embodiments, an RNAi agent of the invention may contain an ultra-low number of nucleotides containing a 2'-fluoro modification, e.g., 2 or fewer nucleotides containing a 2'-fluoro modification. For example, the RNAi agent may contain 2, 1 of 0 nucleotides with a 2'-fluoro modification. In a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

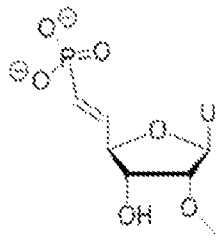


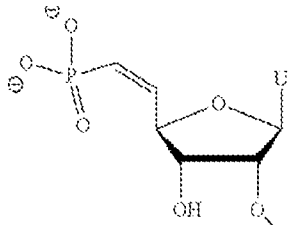
wherein * indicates the location of the bond to 5'-position of the adjacent nucleotide;

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

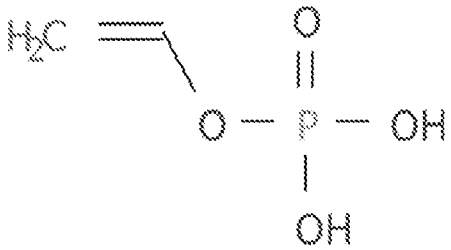
B is a nucleobase or a modified nucleobase, optionally where B is adenine, guanine, cytosine, thymine or uracil.

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-

vinylphosphonate, ), 5′-Z-VP isomer (i.e., cis-vinylphosphonate,

), or mixtures thereof.

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.

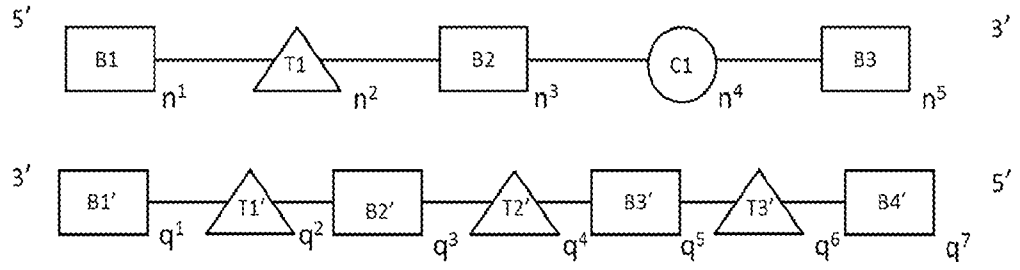
As described in more detail below, the RNAi agent that contains conjugations of one or more carbohydrate moieties to a RNAi agent can optimize one or more properties of the RNAi agent. In many cases, the carbohydrate moiety will be attached to a modified subunit of the RNAi agent. For example, the ribose 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 pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, 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):

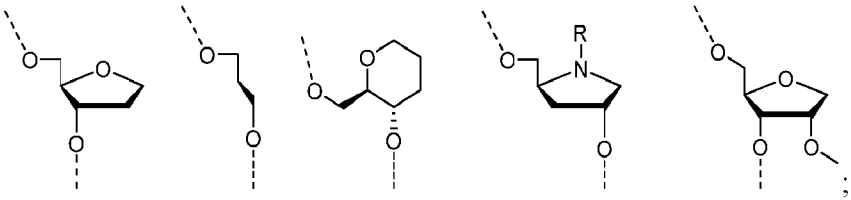



(L),

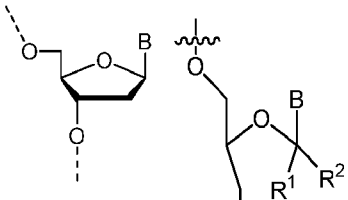
In formula (L), B1, B2, B3, B1', B2', B3', and B4' each are independently a nucleotide containing a modification selected from the group consisting of 2'-O-alkyl, 2'-substituted alkoxy, 2'-substituted alkyl, 2'-halo, ENA, and BNA/LNA. In certain embodiments, B1, B2, B3, B1', B2', B3', and B4' each contain 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:

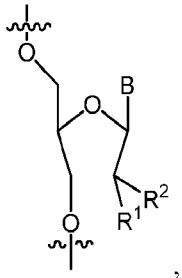


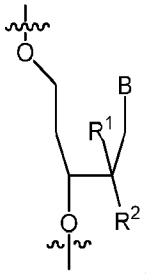
; and iii) sugar modification selected from the group consisting of:

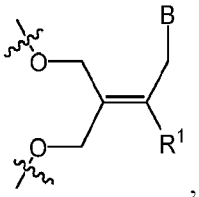


2'-deoxy

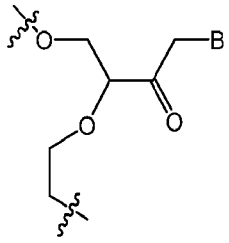




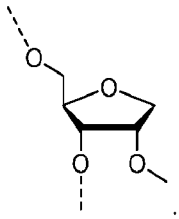




, and



, 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.

$n^4$, $q^2$, 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.

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).

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

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.

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).

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.

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, $n^1$ 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.

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 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, $n^1$ is 6, 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 7, 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.

In certain embodiments, B1 is 2'-OMe or 2'-F, $n^1$ is 6, 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 7, 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 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, $n^1$ 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 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.

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 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, $n^1$ 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 5, T2' is 2'-F, $q^4$ is 1, 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; 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, $n^1$ 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 5, T2' is 2'-F, $q^4$ is 1, 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; 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, $n^1$ 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.

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; 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).

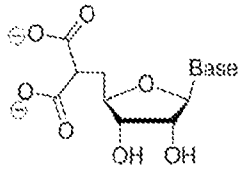
In certain embodiments, B1 is 2'-OMe or 2'-F, $n^1$ is 8, T1 is 2'F, $n^2$ is 3, B2 is 2'-OMe, $n^3$ is 7, $n^4$ is 0, B3 is 2'OMe, $n^5$ is 3, B1' is 2'-OMe or 2'-F, $q^1$ is 9, T1' is 2'-F, $q^2$ is 1, B2' is 2'-OMe or 2'-F, $q^3$ is 4, T2' is 2'-F, $q^4$ is 2, B3' is 2'-OMe or 2'-F, $q^5$ is 5, T3' is 2'-F, $q^6$ is 1, B4' is 2'-F, and $q^7$ is 1.

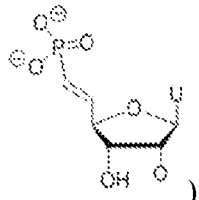
In certain embodiments, B1 is 2'-OMe or 2'-F, $n^1$ is 8, T1 is 2'F, $n^2$ is 3, B2 is 2'-OMe, $n^3$ is 7, $n^4$ is 0, B3 is 2'-OMe, $n^5$ is 3, B1' is 2'-OMe or 2'-F, $q^1$ is 9, T1' is 2'-F, $q^2$ is 1, B2' is 2'-OMe or 2'-F, $q^3$ is 4, T2' is 2'-F, $q^4$ is 2, B3' is 2'-OMe or 2'-F, $q^5$ is 5, T3'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

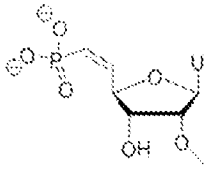
In certain embodiments, B1 is 2'-OMe or 2'-F, $n^1$ is 8, T1 is 2'F, $n^2$ is 3, B2 is 2'-OMe, $n^3$ is 7, $n^4$ is 0, B3 is 2'-OMe, $n^5$ is 3, B1' is 2'-OMe or 2'-F, $q^1$ is 9, T1' is 2'-F, $q^2$ is 1, B2' is 2'-OMe or 2'-F, $q^3$ is 4, $q^4$ is 0, B3' is 2'-OMe or 2'-F, $q^5$ is 7, T3' is 2'-F, $q^6$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

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 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'-$PS_2$), 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'-$PS_2$. In certain embodiments, the RNAi agent comprises a 5'-$PS_2$ in the antisense strand.

In certain embodiments, the RNAi agent comprises a 5'-$PS_2$. In certain embodiments, the RNAi agent comprises a 5'-deoxy-5'-*C*-malonyl in the antisense strand.



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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. 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. 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, 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. 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. The RNAi agent also comprises a 5'- $PS_2$.

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. 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.

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'-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'- $PS_2$.

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, $n^1$ 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'-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'-OMe, and $q^7$ is 1. The RNAi agent also comprises a 5'- $PS_2$.

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'-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; 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.

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; 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.

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; 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, $n^1$ 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; 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'- $PS_2$.

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; 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, $n^1$ 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. 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, 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. 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'-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, 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. The dsRNA RNA agent also comprises a 5'- $PS_2$.

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. 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'-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, 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'- 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'-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, 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'- $PS_2$.

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.

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'- $PS_2$.

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'- $PS_2$.

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.

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'-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 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 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'- $PS_2$ and a targeting ligand. In certain embodiments, the 5'-$PS_2$ 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 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'-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), 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, $n^1$ 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; 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, $n^1$ 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; 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.



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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; 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'-$PS_2$ and a targeting ligand. In certain embodiments, the 5'-$PS_2$ 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'-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), 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, $n^1$ 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'-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, 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'-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'-$PS_2$ and a targeting ligand. In certain embodiments, the 5'-$PS_2$ 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'- $PS_2$ and a targeting ligand. In certain embodiments, the 5'-$PS_2$ 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);

and

(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

(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 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);

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, 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, 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'-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);

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

(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 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

(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 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. Acid. 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-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic 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-κB.

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.



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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).

*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 antennopedia. 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.

An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.*, glyciosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidiomimemtics 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.

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 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).

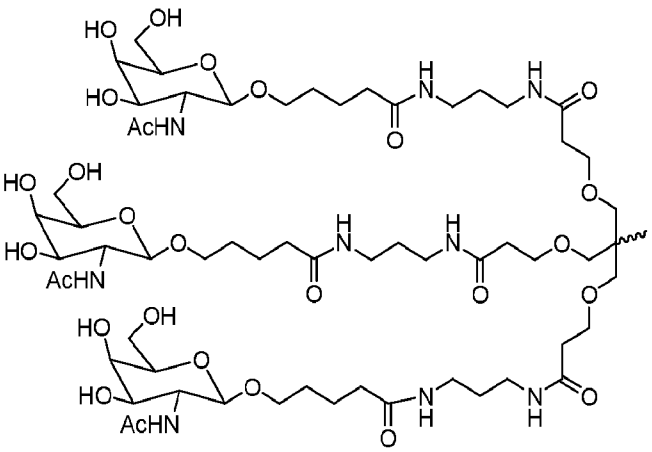
*C. Carbohydrate Conjugates*

In some embodiments of the compositions and methods of the invention, an iRNA oligonucleotide 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 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- and trisaccharides include sugars having two or three monosaccharide units (*e.g.*, C5, C6, C7, or C8).

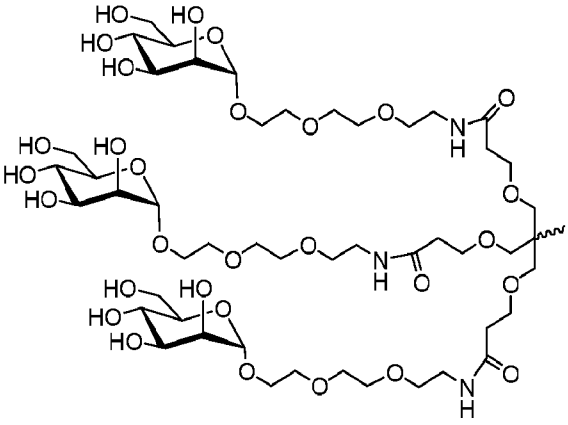
In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



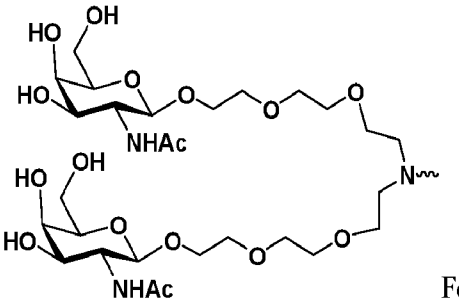
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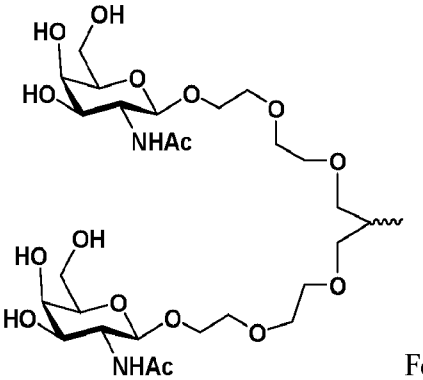
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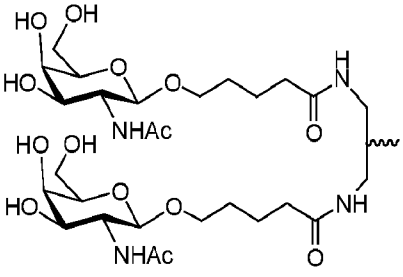
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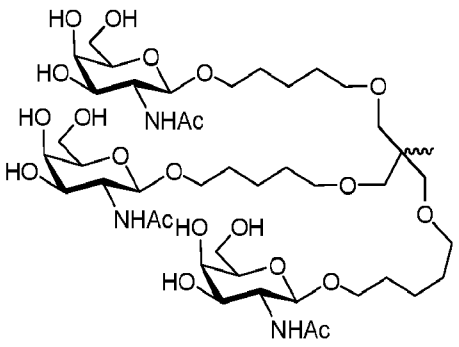
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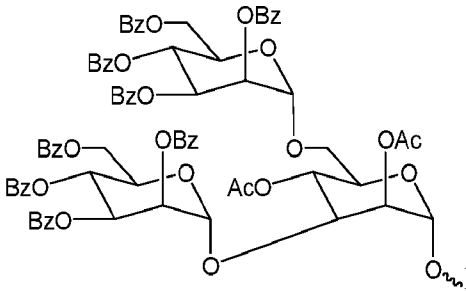
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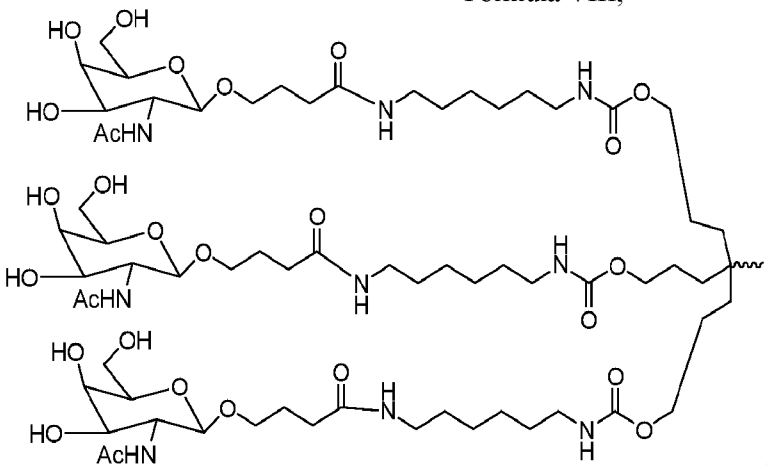
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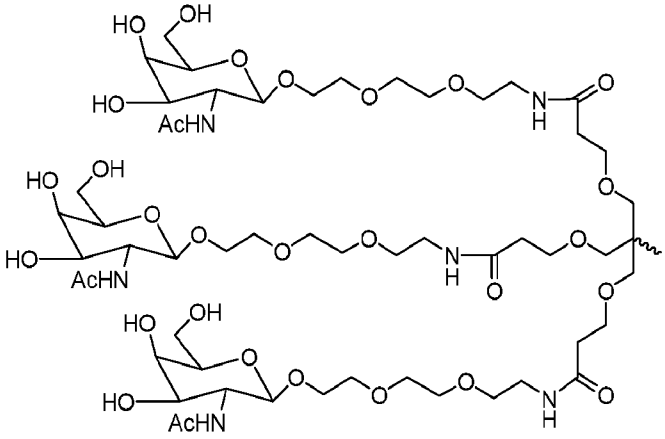
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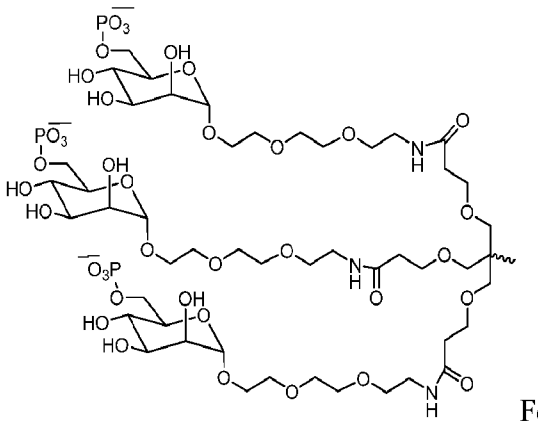
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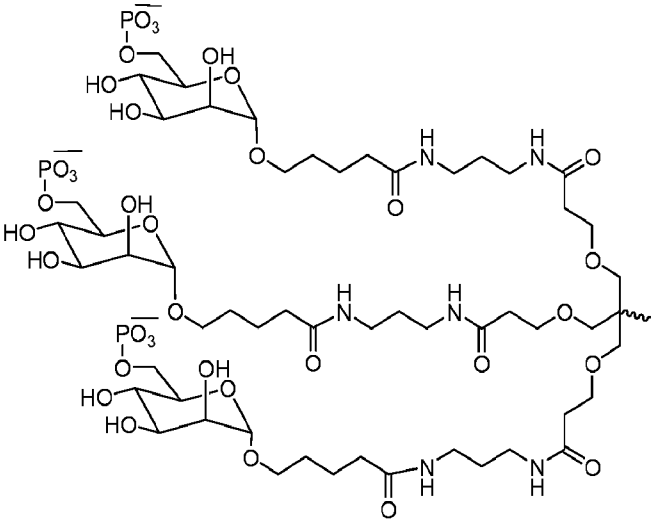
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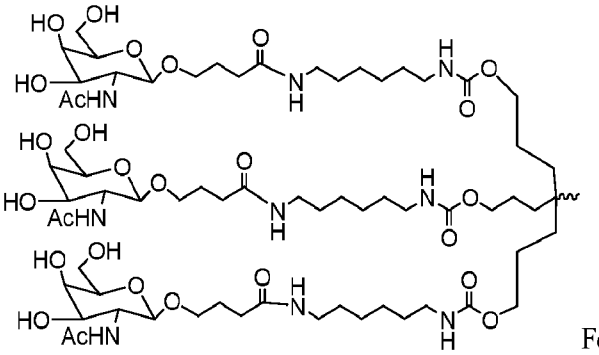
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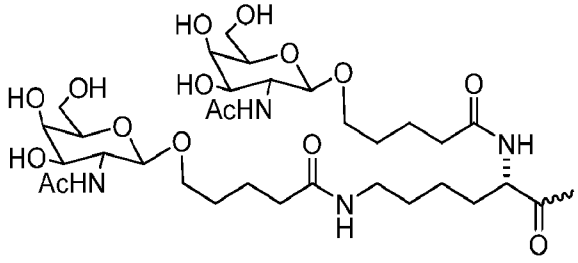
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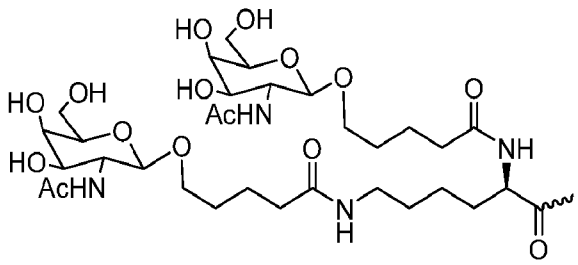
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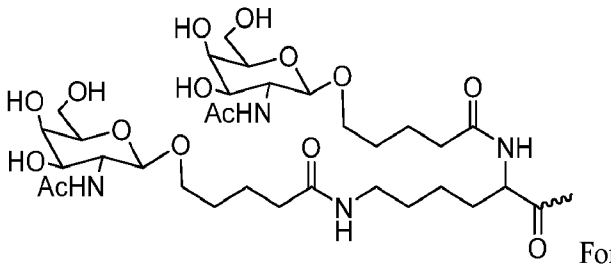
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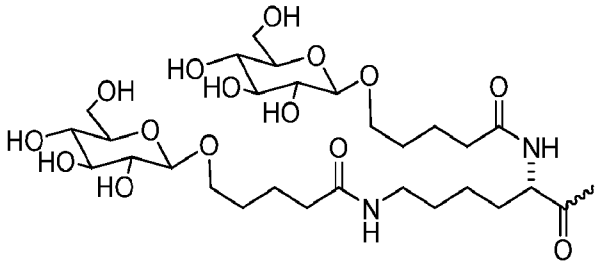
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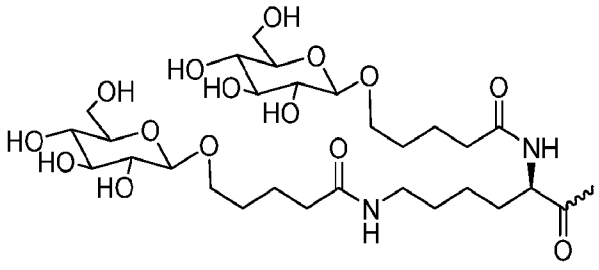
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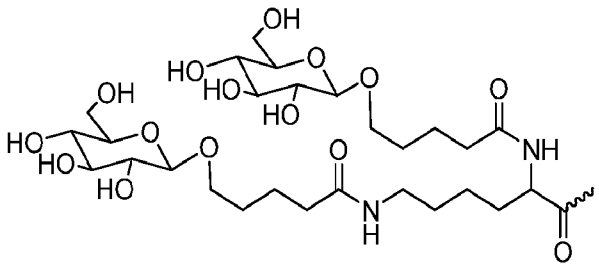
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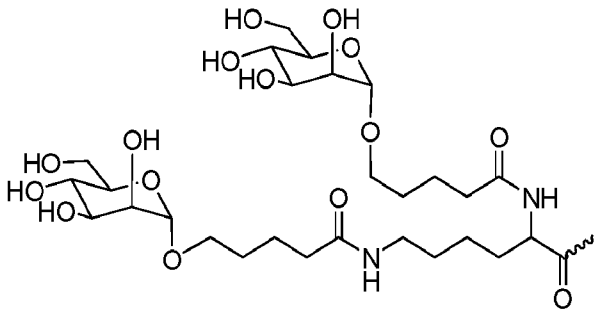
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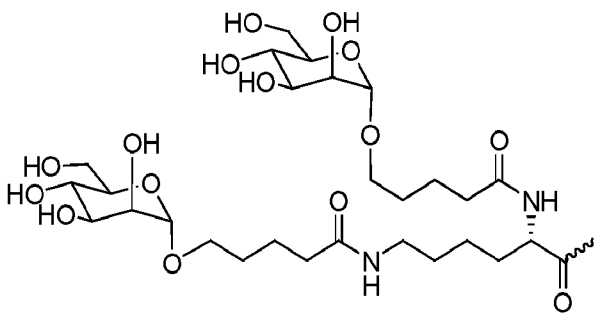
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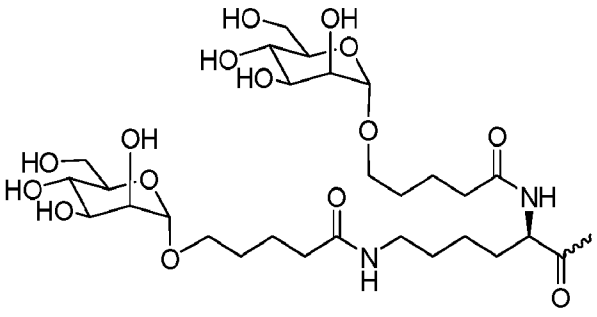
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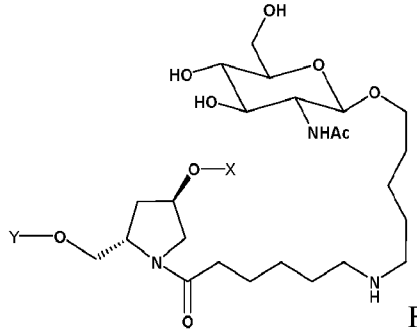
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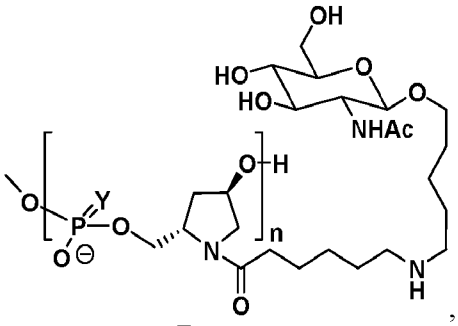
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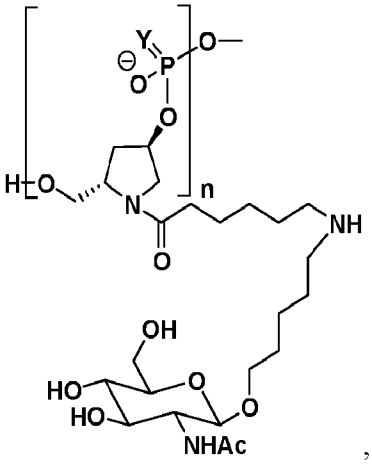
Formula XXII,



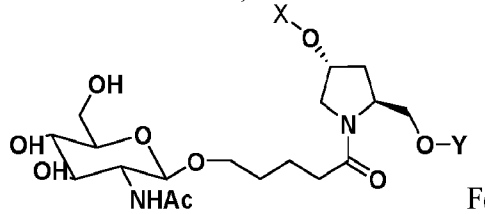
Formula XXIII;



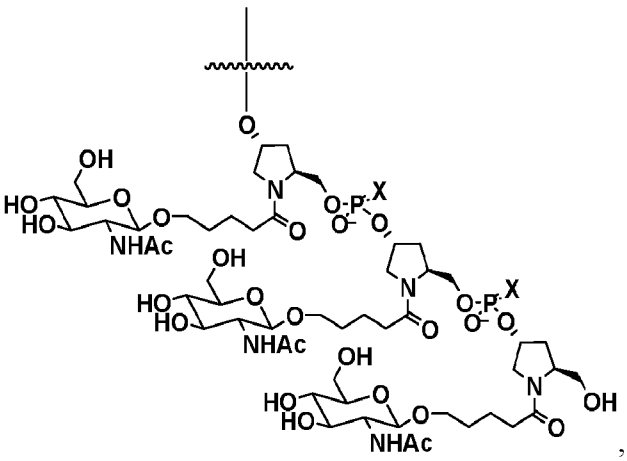
, wherein Y is O or S and n is 3 -6 (Formula XXIV);



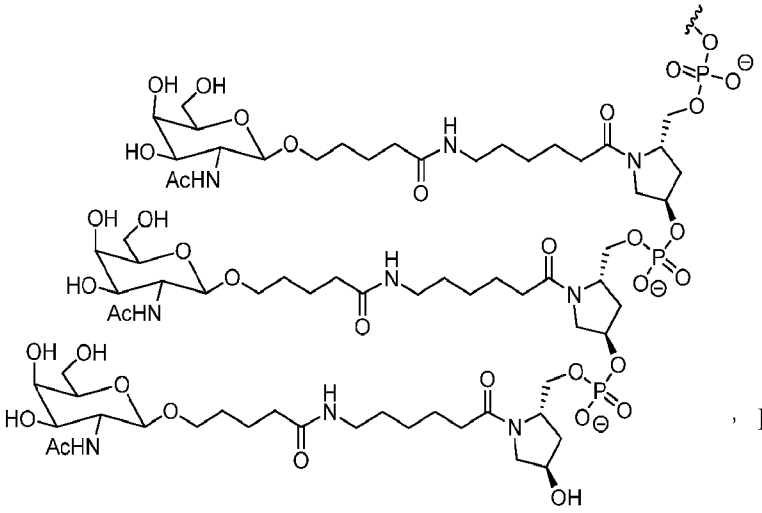
, wherein Y is O or S and n is 3-6 (Formula XXV);



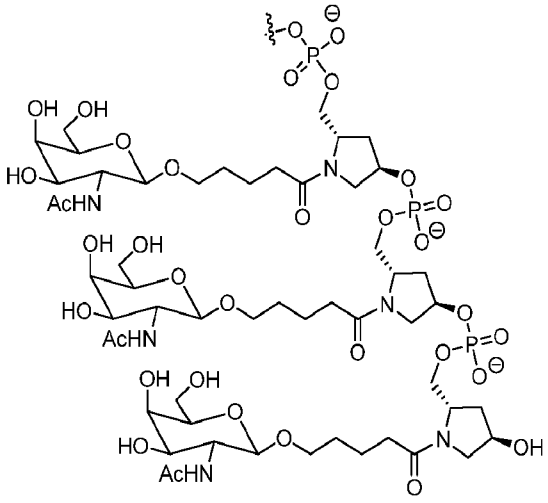
Formula XXVI;



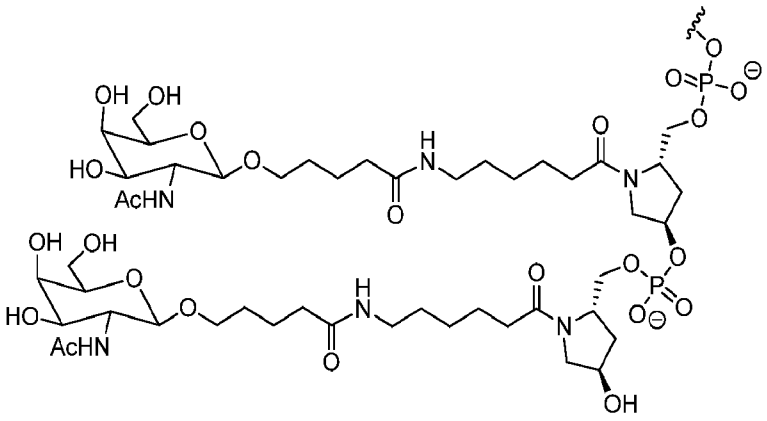
, wherein X is O or S (Formula XXVII);



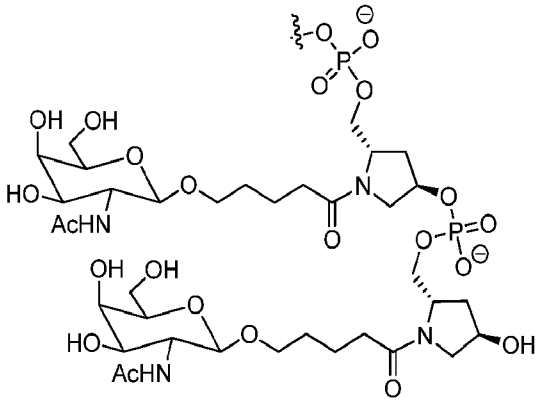
, Formula XXVIII;



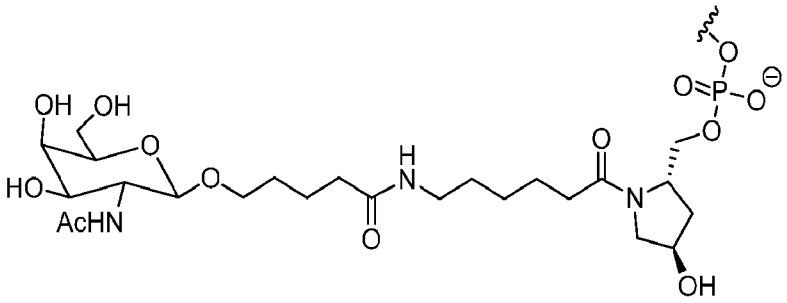
, Formula XXIX;



Formula XXX;

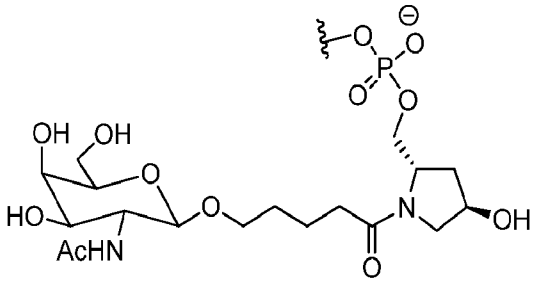


Formula XXXI;

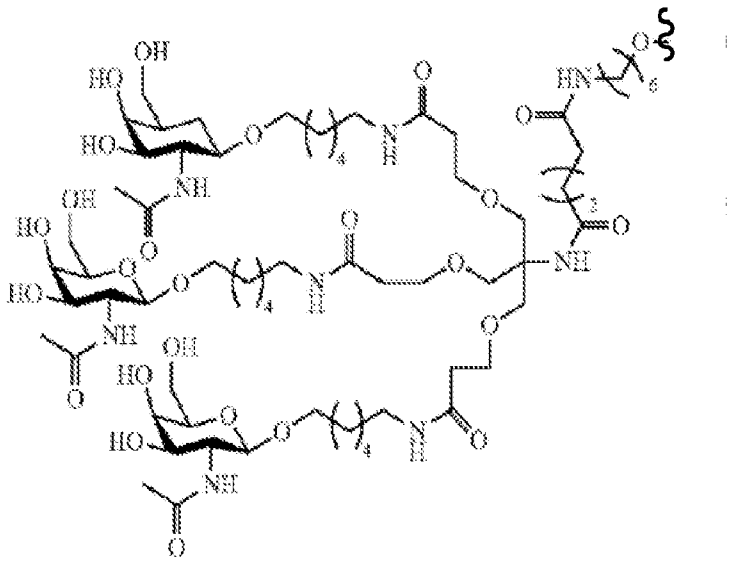


Formula XXXII;

, and

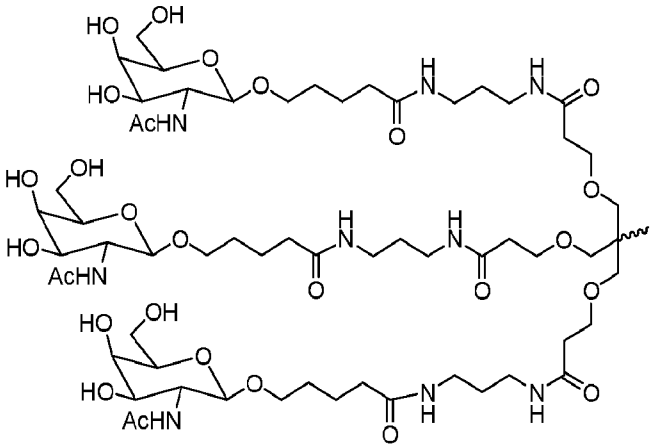


Formula XXXIII.



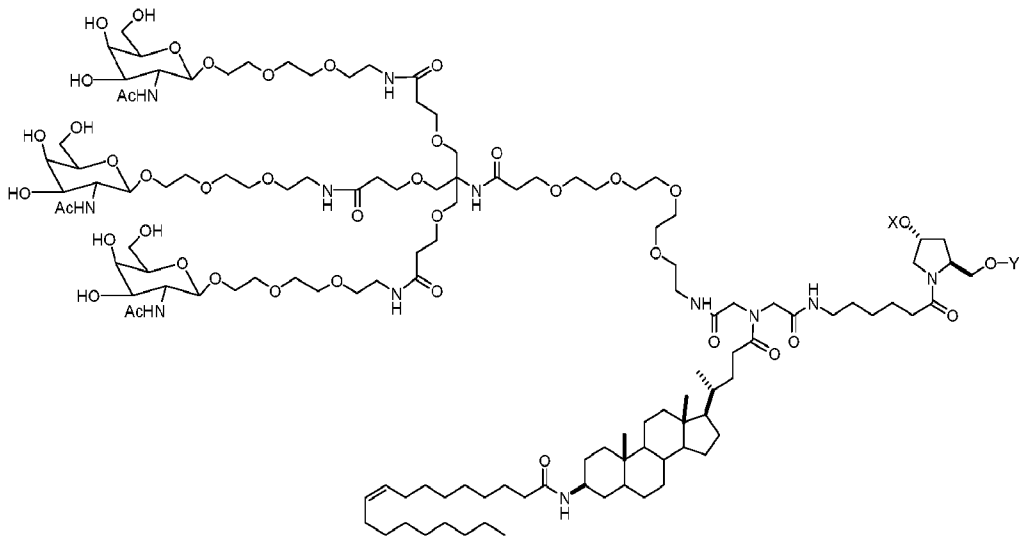
Formula XXXIV.

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, $SO_2$, $SO_2NH$ 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, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), $SO_2$, 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 know 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-, -S-P(O)(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*

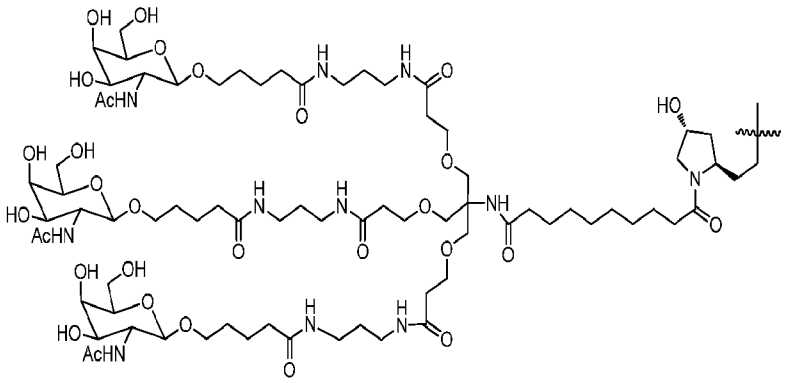
In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides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



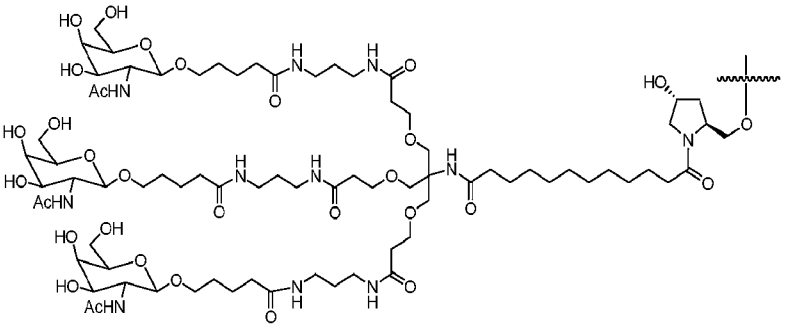
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group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally 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.

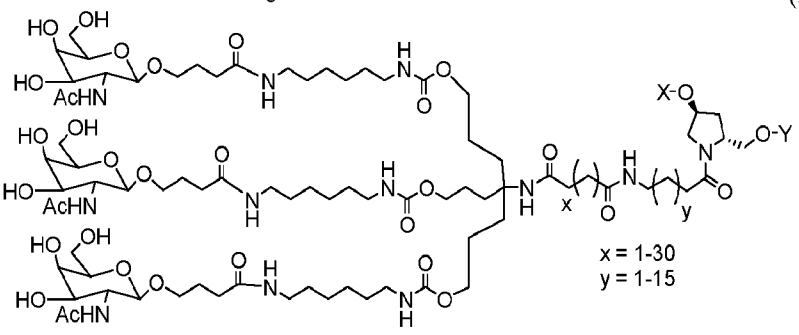
In one embodiment, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



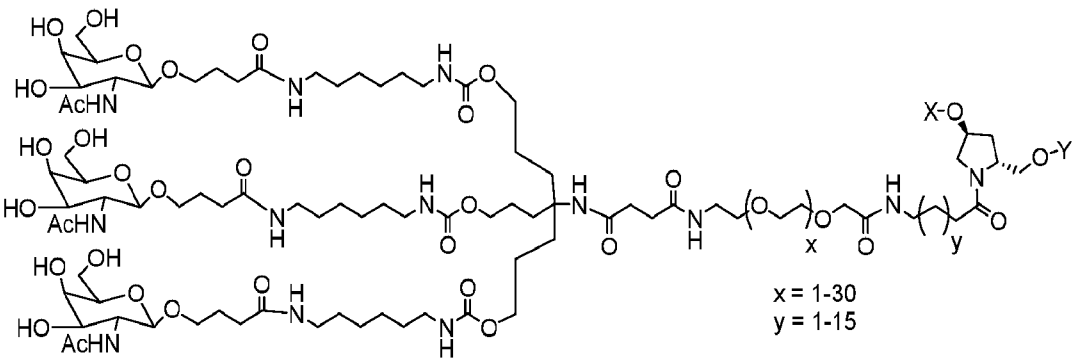
(Formula XXXVI),



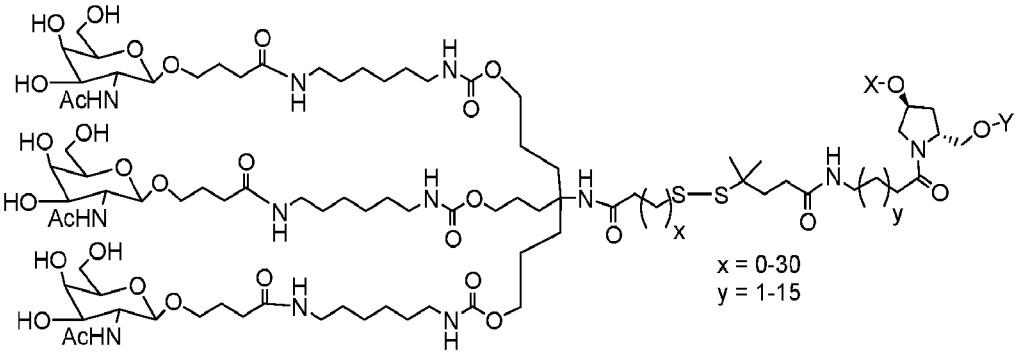
(Formula XXXVII),



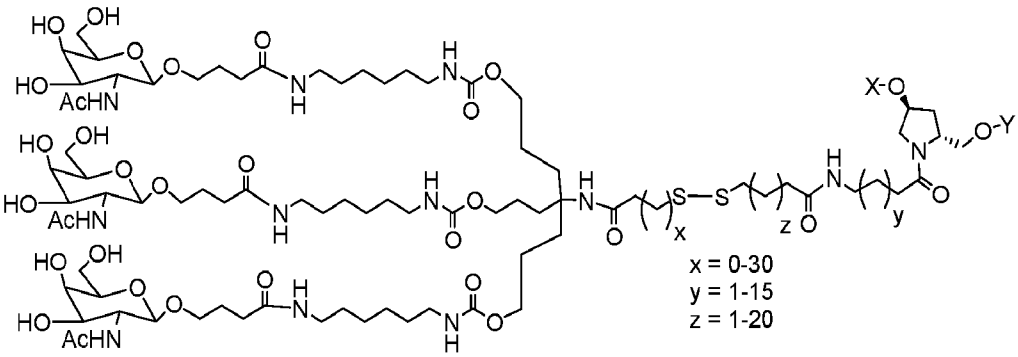

(Formula XXXVIII),



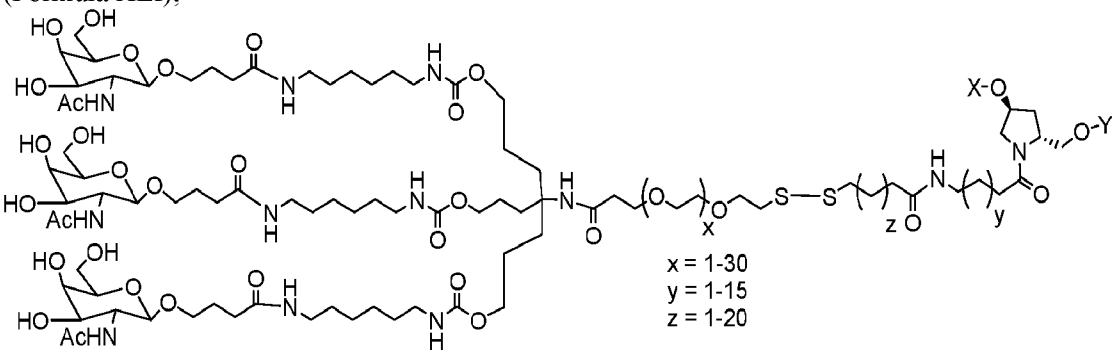

(Formula XXXIX),



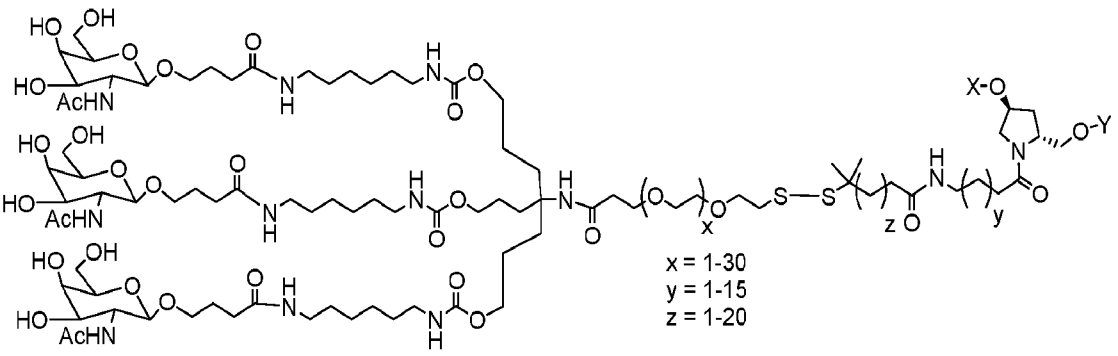

(Formula XL),




(Formula XLI),




(Formula XLII), and

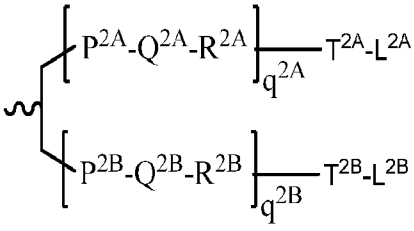



(Formula XLIII), when one of X or Y is an oligonucleotide, the other is a hydrogen.

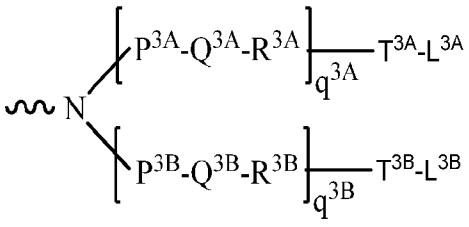
In certain embodiments of the compositions and methods of the invention, a ligand is one or more GalNAc (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

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:

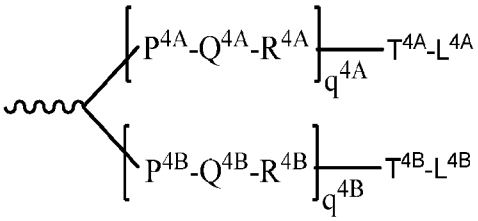
Formula XLIV



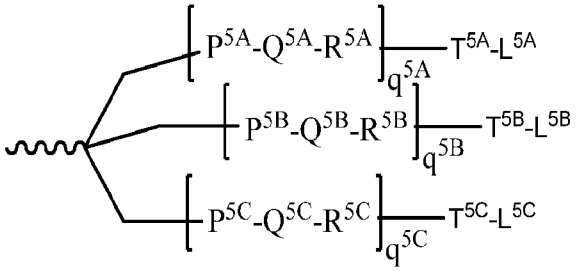

Formula XLV




,




,




;

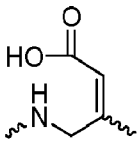
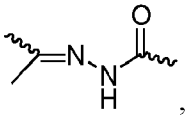
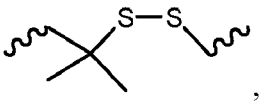
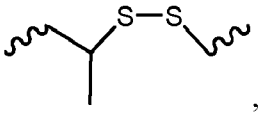
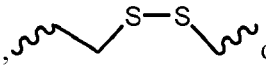
Formula XLVI Formula XLVII

wherein:

q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

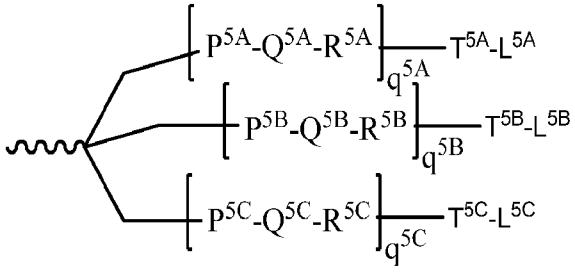
$P^{2A}$, $P^{2B}$, $P^{3A}$, $P^{3B}$, $P^{4A}$, $P^{4B}$, $P^{5A}$, $P^{5B}$, $P^{5C}$, $T^{2A}$, $T^{2B}$, $T^{3A}$, $T^{3B}$, $T^{4A}$, $T^{4B}$, $T^{4A}$, $T^{5B}$, $T^{5C}$ are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), $CH_2$, $CH_2NH$ or $CH_2O$;

$Q^{2A}$, $Q^{2B}$, $Q^{3A}$, $Q^{3B}$, $Q^{4A}$, $Q^{4B}$, $Q^{5A}$, $Q^{5B}$, $Q^{5C}$ 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), $SO_2$, $N(R^N)$, 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, $CH_2$, C(O)O, C(O)NH, NHCH($R^a$)C(O), -C(O)-CH($R^a$)-NH-, CO, CH=N-O, , , , ,  or heterocyclyl;

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

Formula XLVIII



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. Let., 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 CIDEB 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 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 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 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 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 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.

## 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 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.

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, 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.

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 phosphorothiotate 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 phosphorothiotate groups present in the agent.

Pharmaceutical compositions and formulations for topical administration can include transdermal 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 neutral (*e.g.*, dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl 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 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 $C_{1-20}$ 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*).

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 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.

A positively charged synthetic cationic lipid, N-[1-(2,3-dioleyloxy)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 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 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 ("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 dioctaoleoylamide ("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. Transferosomes 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 transferosomes 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 $C_8$ to $C_{22}$ 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-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I -(2,3- dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I -(2,3- dioleyloxy)propyl)-N,N,N-

trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3- dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-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-Dilinoleyloxo-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)ethylazanediyl)didodecan-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.

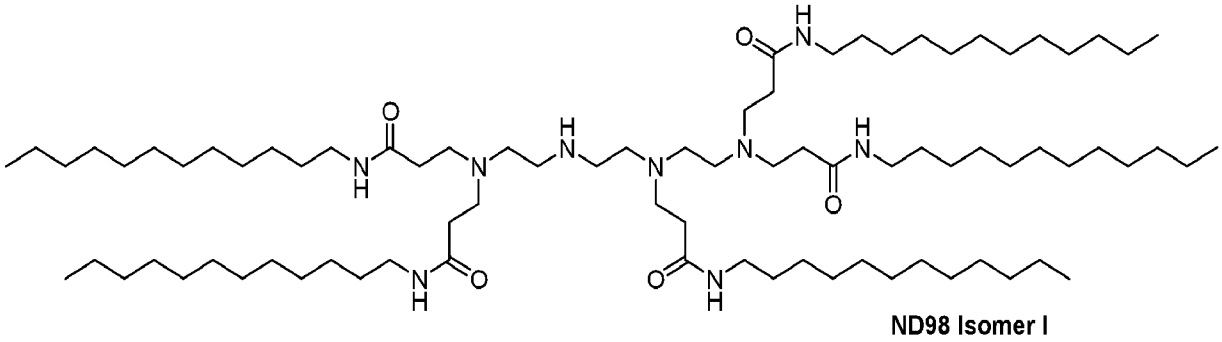
The non-cationic lipid may be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl- phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-l- 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- phosphatidyethanolamine (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-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate may be, for example, a PEG-dilauryloxypropyl ($C_{12}$), a PEG-dimyristyloxypropyl ($C_{14}$), a PEG-dipalmityloxypropyl ($C_{16}$), or a PEG- distearyloxypropyl ($C_{18}$). 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)ethylazanediyl)didodecan-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<br>50/10/38.5/1.5<br>Lipid:siRNA: 7:1 |
| LNP17 | MC3 | MC3/DSPC/Chol/PEG-DSG<br>50/10/38.5/1.5<br>Lipid:siRNA: 10:1 |
| LNP18 | MC3 | MC3/DSPC/Chol/PEG-DMG<br>50/10/38.5/1.5<br>Lipid:siRNA: 12:1 |
| LNP19 | MC3 | MC3/DSPC/Chol/PEG-DMG<br>50/10/35/5<br>Lipid:siRNA: 8:1 |
| LNP20 | MC3 | MC3/DSPC/Chol/PEG-DPG<br>50/10/38.5/1.5<br>Lipid:siRNA: 10:1 |
| LNP21 | C12-200 | C12-200/DSPC/Chol/PEG-DSG<br>50/10/38.5/1.5<br>Lipid:siRNA: 7:1 |
| LNP22 | XTC | XTC/DSPC/Chol/PEG-DSG<br>50/10/38.5/1.5<br>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)

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 (l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO2009/127060, filed April 15, 2009, which is hereby 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. 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 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 minitablets. 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, glucholic 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, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 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, pollulans, 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(isohexylcynaoacrylate), 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, $2C_{1215G}$, 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). Klibanov *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.

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 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 exceeding 0.1μm 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 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 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 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 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 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) 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.*, 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 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 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 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



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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, $C_{1-20}$ 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.*, Touitou, 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), glucholic acid (sodium glucholate), 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; 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, 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 (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 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 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 *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; 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'isothiocyano-stilbene-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 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 agents, *e.g.*, Sodium cellulose phosphate (Calcibind); diuretics, *e.g.*, thiazide diuretics, such as hydrochlorothiazide (Microzide); an insulin sensitizer, such as the PPARγ agonist pioglitazone, a glp-1r agonist, such as liraglutatide, 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 procedures in cell cultures or experimental animals, *e.g.*, for determining the $LD_{50}$ (the dose lethal to 50% of the population) and the $ED_{50}$ (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 $LD_{50}/ED_{50}$. 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 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 $ED_{50}$ 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 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 $IC_{50}$ (*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 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.



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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-butynyl, 2-butynyl, 1-pentynyl, 2-pentynyl, 3-methyl-1 butynyl, 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, tetrahydropyranyl, tetrahydropyridinyl, tetrahydroprimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, tetrahydropyrimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, 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, $-OR^x$, $-NR^xR^y$, $-NR^xC(=O)R^y$, $-NR^xSO_2R^y$, $-C(=O)R^x$, $-C(=O)OR^x$, $-C(=O)NR^xR^y$, $-SO_nR^x$

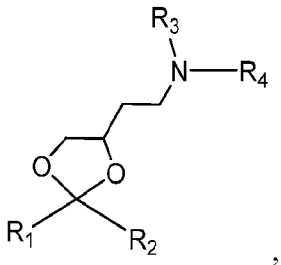
and $-SO_nNR^xR^y$, wherein n is 0, 1 or 2, $R^x$ and $R^y$ are the same or different and independently hydrogen, alkyl or heterocycle, and each of said alkyl and heterocycle substituents may be further substituted with one or more of oxo, halogen, -OH, -CN, alkyl, $-OR^x$, heterocycle, $-NR^xR^y$, $-NR^xC(=O)R^y$, $-NR^xSO_2R^y$, $-C(=O)R^x$, $-C(=O)OR^x$, $-C(=O)NR^xR^y$, $-SO_nR^x$ and $-SO_nNR^xR^y$.

"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, 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 groups can be added and removed using techniques well known in the art.

Synthesis of Formula A:

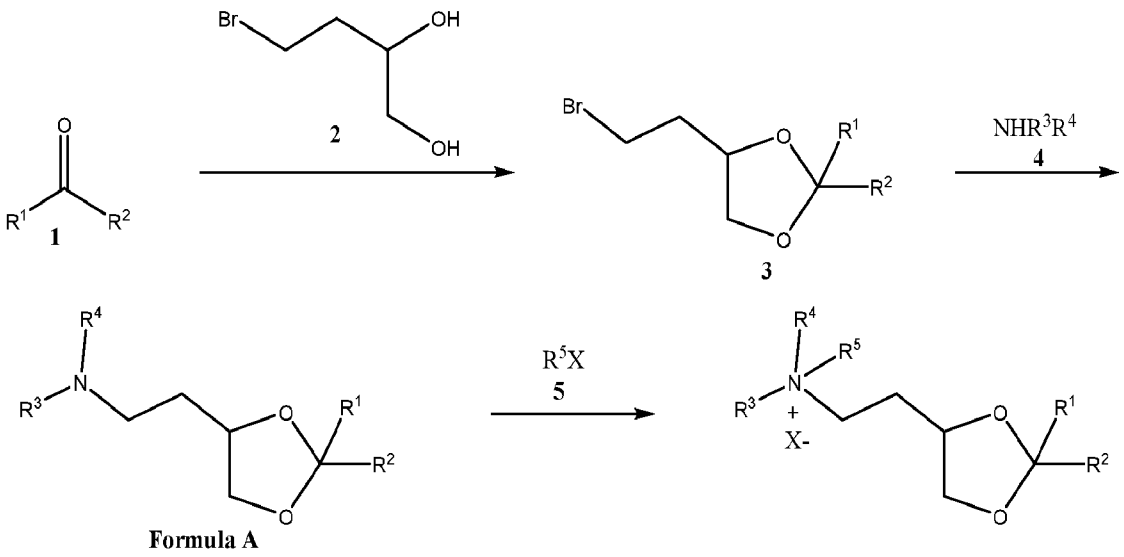
In certain embodiments, nucleic acid-lipid particles featured in the invention are formulated using a cationic lipid of formula A:




,

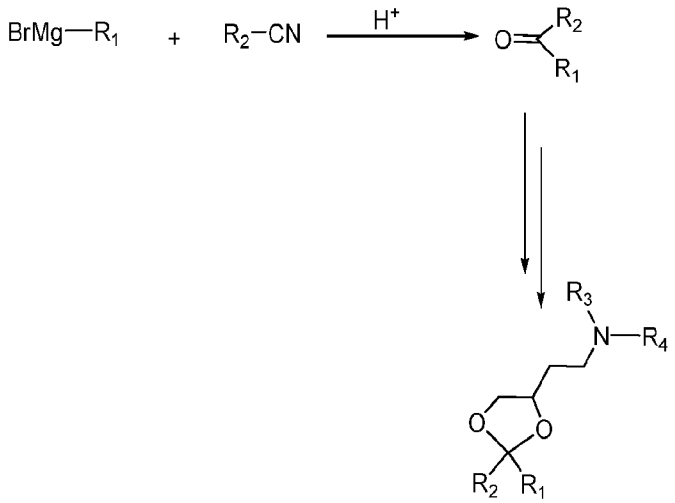
where R1 and R2 are independently alkyl, alkenyl or alkynyl, each can be optionally substituted, and R3 and R4 are independently lower alkyl or R3 and R4 can be taken together to form an optionally substituted heterocyclic ring. In some embodiments, the cationic lipid is XTC (2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane). 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 otherwise.

Scheme 1




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 X is anion counter ion selected from halogen, hydroxide, phosphate, sulfate, or the like.

Scheme 2



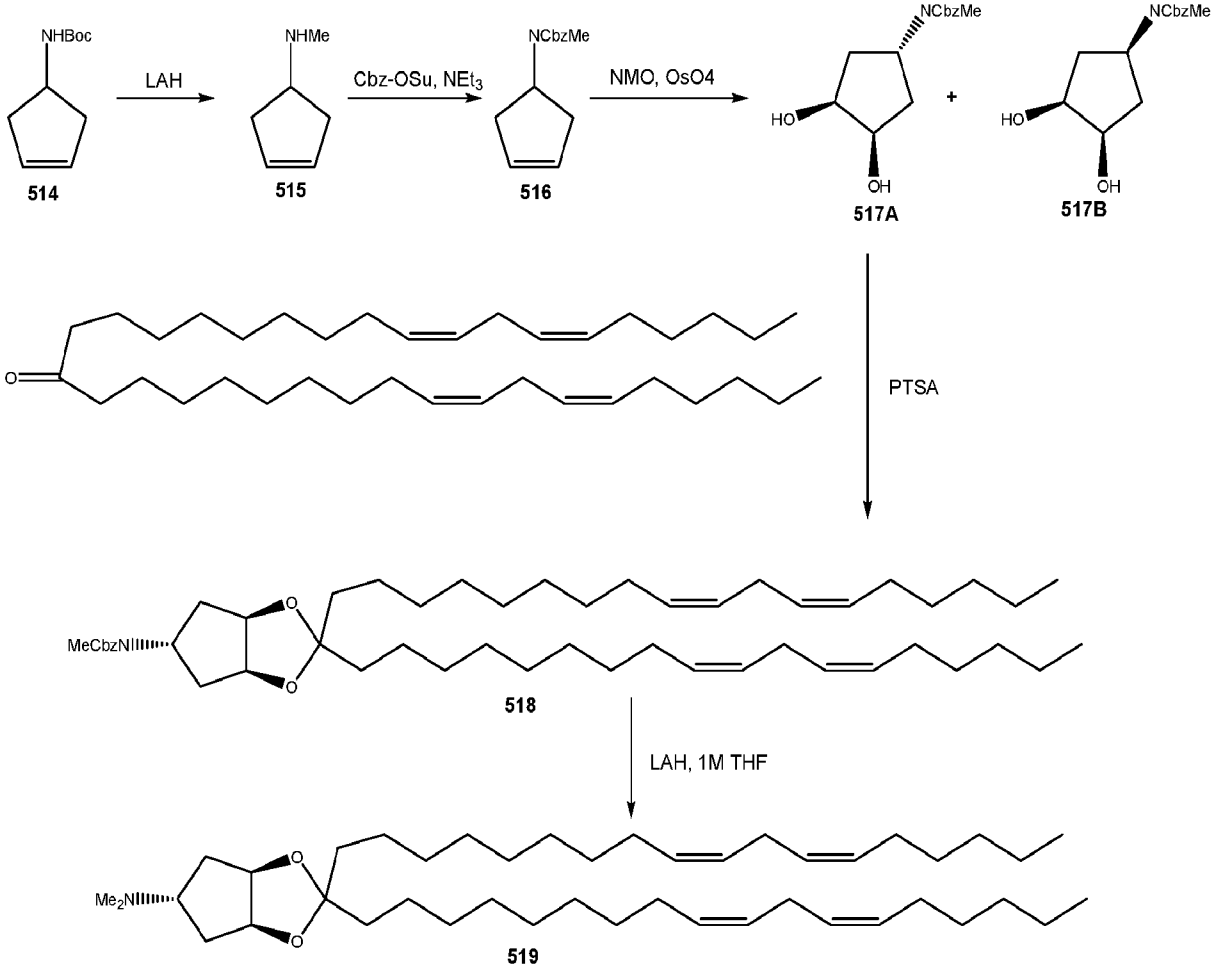

Alternatively, the ketone 1 starting material can be prepared according to Scheme 2. Grignard reagent 6 and cyanide 7 can be purchased or prepared according to methods known to those of ordinary skill in the art. Reaction of 6 and 7 yields ketone 1. Conversion of ketone 1 to the corresponding lipids of formula A is as described in Scheme 1.

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 LiAlH4 (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 0C 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 0C and quenched with careful addition of saturated Na2SO4 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 1H-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 NEt3 (37.2 mL, 0.2669 mol) and cooled to 0 0C 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 NaHCO3 solution (1 x 50 mL). The organic layer was then dried over anhyd. Na2SO4 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%). 1H-NMR (CDCl3, 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 [M+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 OsO4 (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 Na2SO3 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 NaHCO3 (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%). 1H-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:

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:

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 $Na_2SO_4$ 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, 226, 14.1; Electrospray MS (+ve): Molecular weight for C44H80NO2 (M + H)+ Calc. 654.6, Found 654.6.

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. 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 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%.

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.

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.

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.

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 an 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.

Reduction in gene expression can be assessed by any methods known it the art and by methods, *e.g.* qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known it 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 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, 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 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 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 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, *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.

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.

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-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 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.

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-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.

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.

In certain embodiments of the invention the methods may include identifying a subject that would benefit from reduction in CIDEB 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 CIDEB, by determining whether or not a sample from the subject comprises a first nucleic acid encoding a PNPLA3 protein comprising an I148M variation and a second nucleic acid encoding a functional CIDEB protein, and/or a PNPLA3 protein comprising an I144M variation and a functional CIDEB protein, and classifying the subject as a candidate for treating or inhibiting a liver disease by inhibiting CIDEB when both the first and second nucleic acids are detected and/or when both proteins are detected.

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 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 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 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 $kg/m^2$. Obesity 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 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 CIDEB (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 CIDEB (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 CIDEB 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 CIDEB 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, 39, 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, 39, 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. 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 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, *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 (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 dialators; or agents to decrease cholesterol, e.g., statins, selective cholesterol absorption inhibitors, resins; lipid lowering therapies; insulin sensitizers, such as the PPARγ agonist pioglitazone; glp-1r agonists, such as liraglutatide; 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.

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.**

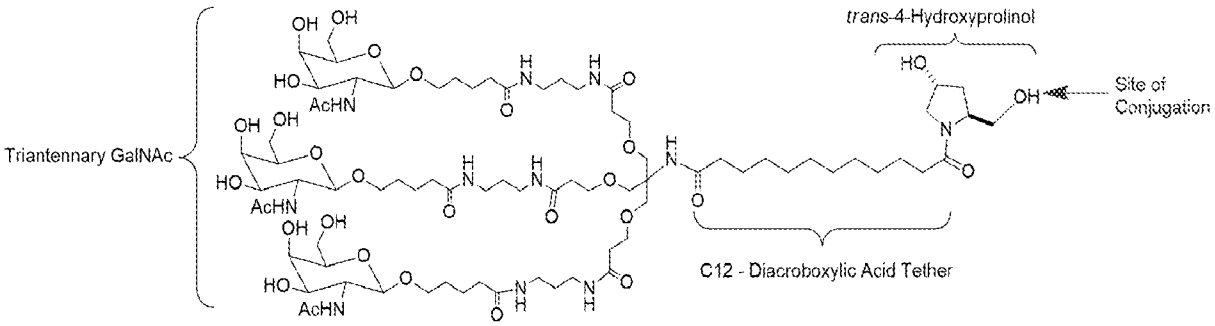
| Abbreviation | Nucleotide(s) |
|---|---|
| A | Adenosine-3'-phosphate |
| Ab | beta-L-adenosine-3`-phosphate |
| Abs | beta-L-adenosine-3`-phosphorothioate |
| Af | 2'-fluoroadenosine-3'-phosphate |
| Afs | 2'-fluoroadenosine-3'-phosphorothioate |

| 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 |

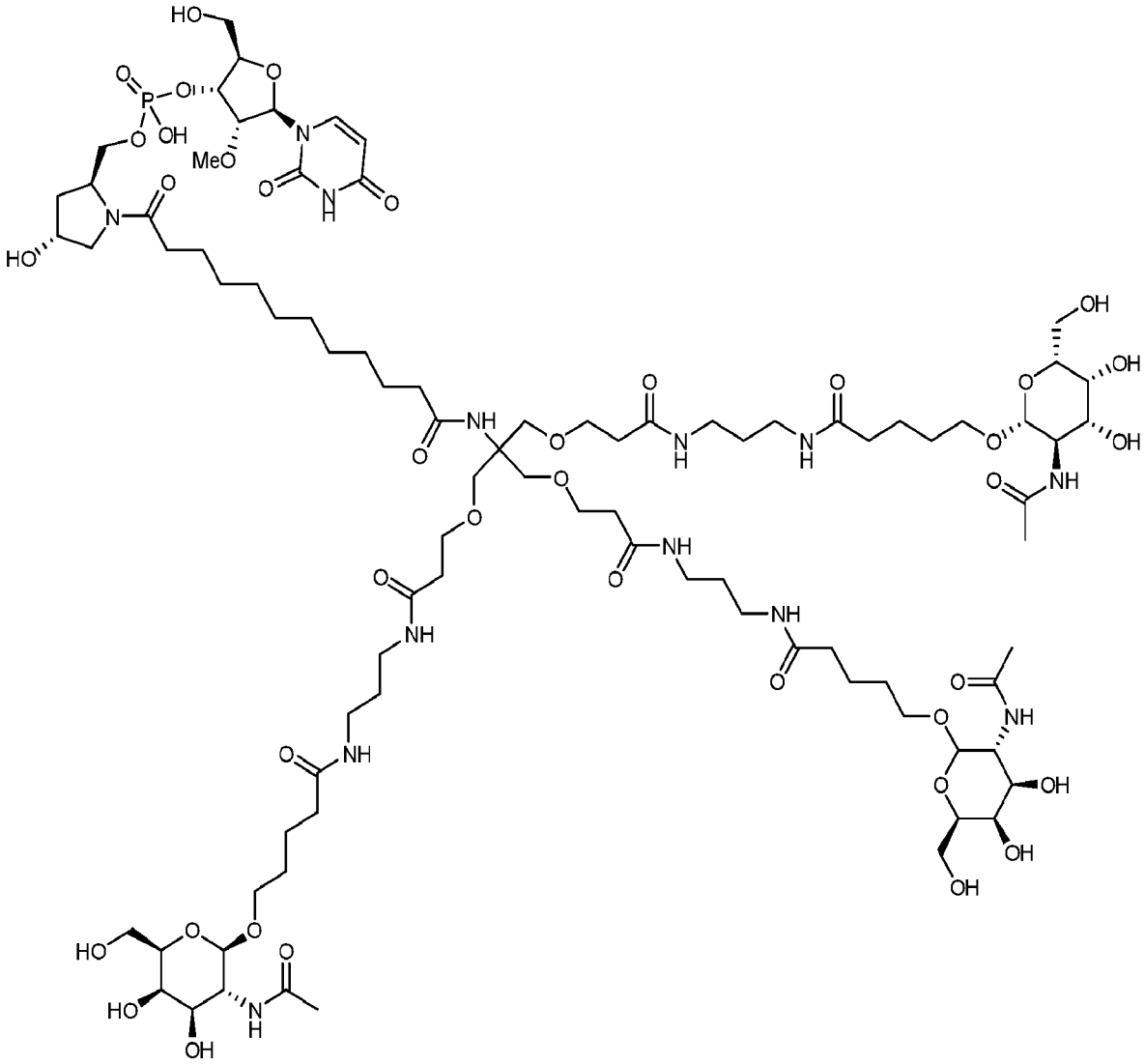
| Abbreviation | Nucleotide(s) |
|---|---|
| g | 2'-O-methylguanosine-3'-phosphate |
| gs | 2'-O-methylguanosine-3'- phosphorothioate |
| t | 2'-O-methyl-5-methyluridine-3'-phosphate |
| ts | 2'-O-methyl-5-methyluridine-3'-phosphorothioate |
| u | 2'-O-methyluridine-3'-phosphate |
| us | 2'-O-methyluridine-3'-phosphorothioate |
| 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]amin 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-tetrahydrofurane-4-methoxy-3-phosphate (abasic 2'-OMe furanose) |
| Y44 | inverted abasic DNA (2-hydroxymethyl-tetrahydrofurane-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 |

[1]The chemical structure of L96 is as follows:




[2]The chemical structure of uL96 is as follows:



## 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*

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*

siRNAs were synthesized and annealed using routine methods known in the art.

Briefly, siRNA sequences were synthesized at 1 μmol scale on a Mermade 192 synthesizer (BioAutomation) using the solid support mediated phosphoramidite chemistry. The solid support was controlled pore glass (500 A) 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 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 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 support and deprotected in sealed 96 deep well plates using 200 μL 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, 200uL of dimethyl sulfoxide (DMSO) and 300ul TEA.3HF reagent was added and the solution was incubated for additional 20min at 60°C. At the end of cleavage and deprotection step, the synthesis plate was allowed to come to room temperature and was precipitated by addition of 1mL of acetontile: 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 20mM 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 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 labled 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 CIDEB 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 GCCCGAAAGU | 11 | 175-195 | ACUUUCGGGCUG AUGCUCGGCUG | 146 | 173-195 |
| AD-1685228 | GCCCGAAAGGA 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 CUGACCCGGCA | 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 | CCUGUGGCCCG 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 | AAUCUGGGUUCC AAGAAGGAGUU | 168 | 850-872 |
| AD-1685614 | UGCUCCCAGUC AGUUGACCUU | 34 | 876-896 | AAGGUCAACUGA CUGGGAGCAGG | 169 | 874-896 |
| AD-1685627 | CCUCCUUCCCA 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 | ACAAUCCCUGCU UUCCUGCCAAG | 173 | 974-996 |
| AD-1685673 | AGCAGGGAUUG UGUUCAUUUU | 39 | 985-1005 | AAAAUGAACACA AUCCCUGCUUU | 174 | 983-1005 |
| AD-1685689 | AUUUGAGGGUU UCACUGUCAU | 40 | 1001-1021 | AUGACAGUGAAA CCCUCAAAUGA | 175 | 999-1021 |
| AD-1685699 | UUCACUGUCAG UGAGAGUCUU | 41 | 1011-1031 | AAGACUCUCACU GACAGUGAAAC | 176 | 1009-1031 |
| AD-1685708 | AGUGAGAGUCU CAGCUUCCAU | 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 UCCACUAAUU | 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 CUGGAAAAUU | 56 | 1207-1227 | AAUUUUCCAGAG 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 | AUUUCCUUCCUC 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 | AUUUUCUGUUAC 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 | AUUCCCUGGAAC UUCUGGGCUGG | 200 | 1337-1359 |
| AD-1685969 | CAGGGAAGGAA CUCUCCGGUU | 66 | 1352-1372 | AACCGGAGAGUU CCUUCCCUGGA | 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 CCUGAGUAAGU | 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 | UCUGGACCUCA 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 | AAGCACUCCAUU CAGCAGUAGGG | 216 | 1573-1595 |
| AD-1686159 | GGAGUGCUAAC CCUGGUGCUU | 82 | 1587-1607 | AAGCACCAGGGU UAGCACUCCAU | 217 | 1585-1607 |
| AD-1686171 | CUGGUGCUAGA 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 | UUCCAGCUGCU 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 | AGCCAUAUGACA GCACUCCACUC | 228 | 1712-1734 |
| AD-1686319 | CAAGCACAGCA AGGACAUCGU | 94 | 1751-1771 | ACGAUGUCCUUG CUGUGCUUGGG | 229 | 1749-1771 |
| AD-1686334 | CAUCGCCCGAU 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 | GAAUGUCAAAG 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 GGACCUCCAU | 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 UGGCCCAGGCC | 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 CCUCCAUUCU | 114 | 2010-2030 | AGAAUGGAGGCG GCCCUUCUGCU | 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 UCAAAUUUCU | 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 GCUAGAUAAU | 122 | 2118-2138 | AUUAUCUAGCCU GUACUGUCUGC | 257 | 2116-2138 |
| AD-1686639 | UAGAUAACCCA 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 CACCUUUCUU | 129 | 2238-2258 | AAGAAAGGUGGC UAGGAAAGAAC | 264 | 2236-2258 |
| AD-1686720 | CACCUUUCUGG CCUCCCACUU | 130 | 2249-2269 | AAGUGGGAGGCC AGAAAGGUGGC | 265 | 2247-2269 |
| AD-1686729 | GGCCUCCCACU UGCCCUGAAU | 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 UCCCAGCCUU | 134 | 2301-2321 | AAGGCUGGGAUC AAGAUGCCUGG | 269 | 2299-2321 |
| AD-1686761 | AUCCCAGCCUG 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 UGUCUUCAUU | 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 cugcguuL96 | 277 | asAfscgcAfgUfCfaa ccUfuCfugcagsgsc | 412 | GCCUGCAGAAGGU UGACUGCGUG | 547 |
| AD-1685173 | asgsccgaAfgGfCfAfagc acgauguL96 | 278 | asCfsaucGfuGfCfuu gcCfuUfcggcususg | 413 | CAAGCCGAAGGCA AGCACGAUGG | 548 |
| AD-1685198 | cscsagcgUfcAfCfGfcug uagcaguL96 | 279 | asCfsugcUfaCfAfgc guGfaCfgcuggscsa | 414 | UGCCAGCGUCACG CUGUAGCAGC | 549 |
| AD-1685207 | csgscuguAfgCfAfGfccg agcaucuL96 | 280 | asGfsaugCfuCfGfgc ugCfuAfcagcgsusg | 415 | CACGCUGUAGCAG CCGAGCAUCA | 550 |
| AD-1685217 | gscscgagCfaUfCfAfgcc cgaaaguL96 | 281 | asCfsuuuCfgGfGfcu gaUfgCfucggcsusg | 416 | CAGCCGAGCAUCA GCCCGAAAGG | 551 |
| AD-1685228 | gscscgaAfaGfGfAfagc acgaaauL96 | 282 | asUfsuucGfuGfCfuu ccUfuUfcgggcsusg | 417 | CAGCCCGAAAGGA AGCACGAAAG | 552 |
| AD-1685240 | gscsacgaAfaGfCfGfguc agagucuL96 | 283 | asGfsacuCfuGfAfcc gcUfuUfcgugcsusu | 418 | AAGCACGAAAGCG GUCAGAGUCU | 553 |
| AD-1685250 | gsgsucagAfgUfCfUfcca ggcucauL96 | 284 | asUfsgagCfcUfGfga gaCfuCfugaccsgsc | 419 | GCGGUCAGAGUCU CCAGGCUCAG | 554 |
| AD-1685271 | gscsacagCfuGfGfCfaua cgcgguuL96 | 285 | asAfsccgCfgUfAfug ccAfgCfugugcscsa | 420 | UGGCACAGCUGGC AUACGCGGUC | 555 |
| AD-1685284 | ascsgcggUfcCfCfUfcca cagguguL96 | 286 | asCfsaccUfgUfGfga ggGfaCfcgcgusasu | 421 | AUACGCGGUCCCU CCACAGGUGG | 556 |
| AD-1685366 | gscsuggcGfuAfCfAfug cugagcguL96 | 287 | asCfsgcuCfaGfCfau guAfcGfccagcsgsu | 422 | ACGCUGGCGUACA UGCUGAGCGC | 557 |
| AD-1685379 | csusgagcGfcGfCfAfcac guaguauL96 | 288 | asUfsacuAfcGfUfgu gcGfcGfcucagscsa | 423 | UGCUGAGCGCGCA CACGUAGUAC | 558 |
| AD-1685388 | csascacgUfaGfUfAfcac cgccuuuL96 | 289 | asAfsaggCfgGfUfgu acUfaCfgugugcsg | 424 | CGCACACGUAGUA CACCGCCUUG | 559 |
| AD-1685406 | cscsggguCfaGfGfAfagg ccacaauL96 | 290 | asUfsuguGfgCfCfuu ccUfgAfcccggscsa | 425 | UGCCGGGUCAGGA AGGCCACAAA | 560 |
| AD-1685418 | gsgsccacAfaAfGfAfgcg gcgugauL96 | 291 | asUfscacGfcCfGfcu cuUfuGfuggccsusu | 426 | AAGGCCACAAAGA GCGGCGUGAG | 561 |
| AD-1685469 | cscsacacCfaCfGfAfagc cguugcuL96 | 292 | asGfscaaCfgGfCfuu cgUfgGfuguggsasg | 427 | CUCCACACCACGA AGCCGUUGCC | 562 |
| AD-1685491 | asgscaggAfaGfGfCfugu gccuguuL96 | 293 | asAfscagGfcAfCfag ccUfuCfcugcusgsc | 428 | GCAGCAGGAAGGC UGUGCCUGUG | 563 |
| AD-1685503 | cscsugugGfcCfCfGfcga agucuuuL96 | 294 | asAfsagaCfuUfCfgc ggGfcCfacaggscsa | 429 | UGCCUGUGGCCCG 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 | csgscgaaGfuCfUfUfccagcucaguL96 | 295 | asCfsugaGfcUfGfgaagAfcUfucgcgsgsg | 430 | CCCGCGAAGUCUUCCAGCUCAGC | 565 |
| AD-1685528 | uscsagcaGfuGfUfCfucguucccuuL96 | 296 | asAfsgggAfaCfGfagacAfcUfgcugasgsc | 431 | GCUCAGCAGUGUCUCGUUCCCUG | 566 |
| AD-1685538 | gscsagacCfgAfCfAfuccuucugguL96 | 297 | asCfscagAfaGfGfauguCfgGfucugcsusa | 432 | UAGCAGACCGACAUCCUUCUGGG | 567 |
| AD-1685549 | uscscuucUfgGfGfCfcuacagccuuL96 | 298 | asAfsggcUfgUfAfggccCfaGfaaggasusg | 433 | CAUCCUUCUGGGCCUACAGCCUG | 568 |
| AD-1685561 | usascagcCfuGfCfCfucuuuucuguL96 | 299 | asCfsagaAfaAfGfaggcAfgGfcuguasgsg | 434 | CCUACAGCCUGCCUCUUUUCUGC | 569 |
| AD-1685570 | cscsucuuUfuCfUfGfccugggaguuL96 | 300 | asAfscucCfcAfGfgcagAfaAfagaggscsa | 435 | UGCCUCUUUUCUGCCUGGGAGUC | 570 |
| AD-1685587 | asgsuccuGfaCfUfUfccacgaggauL96 | 301 | asUfsccuCfgUfGfgaagUfcAfggacuscsc | 436 | GGAGUCCUGACUUCCACGAGGAC | 571 |
| AD-1685599 | csuscaaaCfaCfAfAfcuccuucuuuL96 | 302 | asAfsagaAfgGfAfguugUfgUfuugagsgsu | 437 | ACCUCAAACACAACUCCUUCUUG | 572 |
| AD-1685610 | csusccuuCfuUfGfGfaaccagauuL96 | 303 | asAfsucuGfgGfUfuccaAfgAfaggagsusu | 438 | AACUCCUUCUUGGAACCCAGAUC | 573 |
| AD-1685614 | usgscuccCfaGfUfCfaguugaccuuL96 | 304 | asAfsgguCfaAfCfugacUfgGfgagcasgsg | 439 | CCUGCUCCCAGUCAGUUGACCUG | 574 |
| AD-1685627 | cscsuccuUfcCfCfAfgagcucaguuL96 | 305 | asAfscugAfgCfUfcuggGfaAfggaggscsc | 440 | GGCCUCCUUCCCAGAGCUCAGUG | 575 |
| AD-1685637 | asgsagcuCfaGfUfGfgacacagaauL96 | 306 | asUfsucuGfuGfUfccacUfgAfgcucusgsg | 441 | CCAGAGCUCAGUGGACACAGAAA | 576 |
| AD-1685648 | ascsccuaCfaAfGfGfaucccuuggcuL96 | 307 | asGfsccaAfgGfAfuccuUfgUfaggguscsc | 442 | GGACCCUACAAGGAUCCUUGGCA | 577 |
| AD-1685664 | usgsgcagGfaAfAfGfcaggauuguL96 | 308 | asCfsaauCfcCfUfgcuuUfcCfugccasasg | 443 | CUUGGCAGGAAAGCAGGGAUUGU | 578 |
| AD-1685673 | asgscaggGfaUfUfGfuguucauuuuL96 | 309 | asAfsaauGfaAfCfacaaUfcCfcugcususu | 444 | AAAGCAGGGAUUGUGUUCAUUUG | 579 |
| AD-1685689 | asusuugaGfgGfUfUfucacugucauL96 | 310 | asUfsgacAfgUfGfaaacCfcUfcaaausgsa | 445 | UCAUUUGAGGGUUUCACUGUCAG | 580 |
| AD-1685699 | ususcacuGfuCfAfGfugagagucuuL96 | 311 | asAfsgacUfcUfCfacugAfcAfgugaasasc | 446 | GUUUCACUGUCAGUGAGAGUCUC | 581 |
| AD-1685708 | asgsugagAfgUfCfUfcagcuuccauL96 | 312 | asUfsggaAfgCfUfgagaCfuCfucacusgsa | 447 | UCAGUGAGAGUCUCAGCUUCCAU | 582 |
| AD-1685717 | csuscagcUfuCfCfAfugcaacuguuL96 | 313 | asAfscagUfuGfCfauggAfaGfcugagsasc | 448 | GUCUCAGCUUCCAUGCAACUGUC | 583 |
| AD-1685726 | csasugcaAfcUfGfUfccaucacgguL96 | 314 | asCfscguGfaUfGfgacaGfuUfgcaugsgsa | 449 | UCCAUGCAACUGUCCAUCACGGC | 584 |
| AD-1685735 | gsusccauCfaCfGfGfcugcaacuguL96 | 315 | asCfsaguUfgCfAfgccgUfgAfuggacsasg | 450 | CUGUCCAUCACGGCUGCAACUGA | 585 |
| AD-1685744 | gsgscugcAfaCfUfGfaaaucagaguL96 | 316 | asCfsucuGfaUfUfucagUfuGfcagccsgsu | 451 | ACGGCUGCAACUGAAAUCAGAGC | 586 |
| AD-1685770 | csascagcGfcAfCfCfagaagcuaauL96 | 317 | asUfsuagCfuUfCfugguGfcGfcugugsusc | 452 | GACACAGCGCACCAGAAGCUAAA | 587 |
| AD-1685779 | cscsagaaGfcUfAfAfagucuugauuL96 | 318 | asAfsucaAfgAfCfuuuaGfcUfucuggsusg | 453 | CACCAGAAGCUAAAGUCUUGAUG | 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 | asasagucUfuGfAfUfgccaucaaauL96 | 319 | asUfsuugAfuGfGfcaucAfaGfacuuusasg | 454 | CUAAAGUCUUGAUGCCAUCAAAG | 589 |
| AD-1685798 | usgsccauCfaAfAfGfgacaucccuuL96 | 320 | asAfsgggAfuGfUfccuuUfgAfuggcasusc | 455 | GAUGCCAUCAAAGGACAUCCCUG | 590 |
| AD-1685810 | uscsucugUfcAfCfGfuccacuaauuL96 | 321 | asAfsuuaGfuGfGfacguGfaCfagagasusg | 456 | CAUCUCUGUCACGUCCACUAAUC | 591 |
| AD-1685826 | usasaucgGfcAfAfAfaggagaaaauL96 | 322 | asUfsuuuCfuCfCfuuuuGfcCfgauuasgsu | 457 | ACUAAUCGGCAAAAGGAGAAAAG | 592 |
| AD-1685838 | asasgugaGfaGfAfAfgaugaccuauL96 | 323 | asUfsaggUfcAfUfcuucUfcUfcacuususu | 458 | AAAAGUGAGAGAAGAUGACCUAA | 593 |
| AD-1685847 | asasgaugAfcCfUfAfagugugacuuL96 | 324 | asAfsgucAfcAfCfuuagGfuCfaucuuscsu | 459 | AGAAGAUGACCUAAGUGUGACUG | 594 |
| AD-1685856 | usasagugUfgAfCfUfgcagcaggcuL96 | 325 | asGfsccuGfcUfGfcaguCfaCfacuuasgsg | 460 | CCUAAGUGUGACUGCAGCAGGCA | 595 |
| AD-1685869 | asgscaggCfaGfCfUfcuggaaaauuL96 | 326 | asAfsuuuUfcCfAfgagcUfgCfcugcusgsc | 461 | GCAGCAGGCAGCUCUGGAAAAUG | 596 |
| AD-1685878 | csuscuggAfaAfAfUfgaagccagauL96 | 327 | asUfscugGfcUfUfcauuUfuCfcagagscsu | 462 | AGCUCUGGAAAAUGAAGCCAGAG | 597 |
| AD-1685889 | gsasagccAfgAfGfCfagugagccauL96 | 328 | asUfsggcUfcAfCfugcuCfuGfgcuucsasu | 463 | AUGAAGCCAGAGCAGUGAGCCAG | 598 |
| AD-1685898 | cscsgaccAfaGfGfAfggaaggaaauL96 | 329 | asUfsuucCfuUfCfcuccUfuGfgucggsasg | 464 | CUCCGACCAAGGAGGAAGGAAAG | 599 |
| AD-1685902 | gsasggaaGfgAfAfAfgagcagaucuL96 | 330 | asGfsaucUfgCfUfcuuuCfcUfuccucscsu | 465 | AGGAGGAAGGAAAGAGCAGAUCC | 600 |
| AD-1685912 | asgsagcaGfaUfCfCfcaggguuuguuL96 | 331 | asAfscaaAfcCfUfgggaUfcUfgcucususu | 466 | AAAGAGCAGAUCCCAGGUUUGUA | 601 |
| AD-1685921 | cscscaggUfuUfGfUfaacagaaaauL96 | 332 | asUfsuuuCfuGfUfuacaAfaCfcugggsasu | 467 | AUCCCAGGUUUGUAACAGAAAAC | 602 |
| AD-1685931 | usasacagAfaAfAfCfaccacuaaauL96 | 333 | asUfsuuaGfuGfGfuguuUfuCfuguuascsa | 468 | UGUAACAGAAAACACCACUAAAG | 603 |
| AD-1685935 | csasgcacAfgGfAfGfagaaccaccuL96 | 334 | asGfsgugGfuUfCfucucCfuGfugcugsgsg | 469 | CCCAGCACAGGAGAGAACCACCC | 604 |
| AD-1685956 | asgscccaGfaAfGfUfuccagggaauL96 | 335 | asUfsuccCfuGfGfaacuUfcUfgggcusgsg | 470 | CCAGCCCAGAAGUUCCAGGGAAG | 605 |
| AD-1685969 | csasgggaAfgGfAfAfcuccuccgguuL96 | 336 | asAfsccgGfaGfAfguucCfuUfcccugsgsa | 471 | UCCAGGGAAGGAACUCUCCGGUC | 606 |
| AD-1685983 | uscscgguCfcAfCfCfauggaguacuL96 | 337 | asGfsuacUfcCfAfugguGfgAfccggasgsa | 472 | UCUCCGGUCCACCAUGGAGUACC | 607 |
| AD-1685994 | asusggagUfaCfCfUfcucagcucuuL96 | 338 | asAfsgagCfuGfAfgaggUfaCfuccausgsg | 473 | CCAUGGAGUACCUCUCAGCUCUG | 608 |
| AD-1686007 | ususacucAfgGfUfCfaguaucuaauL96 | 339 | asUfsuagAfuAfCfugacCfuGfaguaasgsu | 474 | ACUUACUCAGGUCAGUAUCUAAU | 609 |
| AD-1686016 | uscsaguaUfcUfAfAfuauaagcucuL96 | 340 | asGfsagcUfuAfUfauuaGfaUfacugascsc | 475 | GGUCAGUAUCUAAUAUAAGCUCG | 610 |
| AD-1686028 | asusaagcUfcGfGfAfguuuggacguL96 | 341 | asCfsgucCfaAfAfcuccGfaGfcuuausasu | 476 | AUAUAAGCUCGGAGUUUGGACGG | 611 |
| AD-1686053 | uscsuggaCfcUfCfAfgcuccaccauL96 | 342 | asUfsgguGfgAfGfcugaGfgUfccagascsc | 477 | GGUCUGGACCUCAGCUCCACCAC | 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 | asgscgacCfuUfUfCfcgugucuguuL96 | 343 | asAfscagAfcAfCfggaaAfgGfucgcusgsg | 478 | CCAGCGACCUUUCCGUGUCUGUG | 613 |
| AD-1686066 | uscscgugUfcUfGfUfgaucacaaguL96 | 344 | asCfsuugUfgAfUfcacaGfaCfacggasasa | 479 | UUUCCGUGUCUGUGAUCACAAGC | 614 |
| AD-1686075 | gsusgaucAfcAfAfGfcggaccaucuL96 | 345 | asGfsaugGfuCfCfgcuuGfuGfaucacsasg | 480 | CUGUGAUCACAAGCGGACCAUCC | 615 |
| AD-1686086 | csgsgaccAfuCfCfGfgaaaggccuuL96 | 346 | asAfsggcCfuUfUfccggAfuGfguccgscsu | 481 | AGCGGACCAUCCGGAAAGGCCUG | 616 |
| AD-1686098 | asasaggcCfuGfAfCfagcugccacuL96 | 347 | asGfsuggCfaGfCfugucAfgGfccuuuscsc | 482 | GGAAAGGCCUGACAGCUGCCACC | 617 |
| AD-1686116 | cscsaggaGfcUfGfCfuagccaaaguL96 | 348 | asCfsuuuGfgCfUfagcaGfcUfccuggscsg | 483 | CGCCAGGAGCUGCUAGCCAAAGC | 618 |
| AD-1686126 | csusagccAfaAfGfCfauuggagacuL96 | 349 | asGfsucuCfcAfAfugcuUfuGfgcuagscsa | 484 | UGCUAGCCAAAGCAUUGGAGACC | 619 |
| AD-1686136 | csasuuggAfgAfCfCfcuacugcuguL96 | 350 | asCfsagcAfgUfAfggguCfuCfcaaugscsu | 485 | AGCAUUGGAGACCCUACUGCUGA | 620 |
| AD-1686147 | csusacugCfuGfAfAfuggagugcuuL96 | 351 | asAfsgcaCfuCfCfauucAfgCfaguagsgsg | 486 | CCCUACUGCUGAAUGGAGUGCUA | 621 |
| AD-1686159 | gsgsagugCfuAfAfCfccuggugcuuL96 | 352 | asAfsgcaCfcAfGfgguuAfgCfacuccsasu | 487 | AUGGAGUGCUAACCCUGGUGCUA | 622 |
| AD-1686171 | csusggugCfuAfGfAfggaggaugguL96 | 353 | asCfscauCfcUfCfcucuAfgCfaccagsgsg | 488 | CCCUGGUGCUAGAGGAGGAUGGA | 623 |
| AD-1686181 | asgsgaggAfuGfGfAfacugcaguguL96 | 354 | asCfsacuGfcAfGfuuccAfuCfcuccuscsu | 489 | AGAGGAGGAUGGAACUGCAGUGG | 624 |
| AD-1686197 | asgsuggaCfaGfUfGfaggacuucuuL96 | 355 | asAfsgaaGfuCfCfucacUfgUfccacusgsc | 490 | GCAGUGGACAGUGAGGACUUCUU | 625 |
| AD-1686207 | gsasggacUfuCfUfUfccagcugcuuL96 | 356 | asAfsgcaGfcUfGfgaagAfaGfuccucsasc | 491 | GUGAGGACUUCUUCCAGCUGCUG | 626 |
| AD-1686216 | ususccagCfuGfCfUfggaggaugauL96 | 357 | asUfscauCfcUfCfcagcAfgCfuggaasgsa | 492 | UCUUCCAGCUGCUGGAGGAUGAC | 627 |
| AD-1686225 | csusggagGfaUfGfAfcacgugccuuL96 | 358 | asAfsggcAfcGfUfgucaUfcCfuccagscsa | 493 | UGCUGGAGGAUGACACGUGCCUG | 628 |
| AD-1686242 | cscsugauGfgUfGfUfugcagucuguL96 | 359 | asCfsagaCfuGfCfaacaCfcAfucaggscsa | 494 | UGCCUGAUGGUGUUGCAGUCUGG | 629 |
| AD-1686251 | gsusugcaGfuCfUfGfgucagagcuuL96 | 360 | asAfsgcuCfuGfAfccagAfcUfgcaacsasc | 495 | GUGUUGCAGUCUGGUCAGAGCUG | 630 |
| AD-1686265 | asgsagcuGfgAfGfCfccuacaagguL96 | 361 | asCfscuuGfuAfGfggcuCfcAfgcucusgsa | 496 | UCAGAGCUGGAGCCCUACAAGGA | 631 |
| AD-1686274 | gscsccuaCfaAfGfGfaguggaguguL96 | 362 | asCfsacuCfcAfCfuccuUfgUfagggcsusc | 497 | GAGCCCUACAAGGAGUGGAGUGC | 632 |
| AD-1686286 | gsusggagUfgCfUfGfucauauggcuL96 | 363 | asGfsccaUfaUfGfacagCfaCfuccacsusc | 498 | GAGUGGAGUGCUGUCAUAUGGCC | 633 |
| AD-1686319 | csasagcaCfaGfCfAfaggacaucguL96 | 364 | asCfsgauGfuCfCfuugcUfgUfgcuugsgsg | 499 | CCCAAGCACAGCAAGGACAUCGC | 634 |
| AD-1686334 | csasucgcCfcGfAfUfucaccuuuguL96 | 365 | asCfsaaaGfgUfGfaaucGfgGfcgaugsusc | 500 | GACAUCGCCCGAUUCACCUUUGA | 635 |
| AD-1686343 | asusucacCfuUfUfGfacguguacauL96 | 366 | asUfsguaCfaCfGfucaaAfgGfugaauscsg | 501 | CGAUUCACCUUUGACGUGUACAA | 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 | ascsguguAfcAfAfGfcaaaacccuuL96 | 367 | asAfsgggUfuUfUfgcuuGfuAfcacguscsa | 502 | UGACGUGUACAAGCAAAACCCUC | 637 |
| AD-1686366 | asasaaccCfuCfGfAfgaccucuuuuL96 | 368 | asAfsaagAfgGfUfcucgAfgGfguuuusgsc | 503 | GCAAAACCCUCGAGACCUCUUUG | 638 |
| AD-1686377 | gsasccucUfuUfGfGfcagccugaauL96 | 369 | asUfsucaGfgCfUfgccaAfaGfaggucsusc | 504 | GAGACCUCUUUGGCAGCCUGAAU | 639 |
| AD-1686394 | gsasauguCfaAfAfGfccacauucuuL96 | 370 | asAfsgaaUfgUfGfgcuuUfgAfcauucsasg | 505 | CUGAAUGUCAAAGCCACAUUCUA | 640 |
| AD-1686403 | asgsccacAfuUfCfUfacggggcucuuL96 | 371 | asAfsgagCfcCfGfuagaAfuGfuggcususu | 506 | AAAGCCACAUUCUACGGGCUCUA | 641 |
| AD-1686417 | gsgscucuAfcUfCfUfaugaguuguuL96 | 372 | asAfscaaCfuCfAfuagaGfuAfgagccscsg | 507 | CGGGCUCUACUCUAUGAGUUGUG | 642 |
| AD-1686426 | csusaugaGfuUfGfUfgacuuucaauL96 | 373 | asUfsugaAfaGfUfcacaAfcUfcauagsasg | 508 | CUCUAUGAGUUGUGACUUUCAAG | 643 |
| AD-1686435 | gsusgacuUfuCfAfAfggacuuggcuL96 | 374 | asGfsccaAfgUfCfcuugAfaAfgucacsasa | 509 | UUGUGACUUUCAAGGACUUGGCC | 644 |
| AD-1686451 | usgsgcccAfaAfGfAfaaguacucauL96 | 375 | asUfsgagUfaCfUfuucuUfuGfggccasasg | 510 | CUUGGCCCAAAGAAAGUACUCAG | 645 |
| AD-1686466 | ascsucagGfgAfGfCfuccuucguuuL96 | 376 | asAfsacgAfaGfGfagcuCfcCfugagusasc | 511 | GUACUCAGGGAGCUCCUUCGUUG | 646 |
| AD-1686475 | gscsuccuUfcGfUfUfggaccuccauL96 | 377 | asUfsggaGfgUfCfcaacGfaAfggagcsusc | 512 | GAGCUCCUUCGUUGGACCUCCAC | 647 |
| AD-1686491 | uscscacaCfuGfCfUfgcaaggccuuL96 | 378 | asAfsggcCfuUfGfcagcAfgUfguggasgsg | 513 | CCUCCACACUGCUGCAAGGCCUG | 648 |
| AD-1686508 | cscsugggCfcAfUfAfuguugcugguL96 | 379 | asCfscagCfaAfCfauauGfgCfccaggscsc | 514 | GGCCUGGGCCAUAUGUUGCUGGG | 649 |
| AD-1686518 | asusguugCfuGfGfGfaauuuccucuL96 | 380 | asGfsaggAfaAfUfuccAfgCfaacausasu | 515 | AUAUGUUGCUGGGAAUUUCCUCC | 650 |
| AD-1686531 | ususuccuCfcAfCfCfcuucgucauuL96 | 381 | asAfsugaCfgAfAfggguGfgAfggaaasusu | 516 | AAUUUCCUCCACCCUUCGUCAUG | 651 |
| AD-1686540 | cscscuucGfuCfAfUfgcaguggaguL96 | 382 | asCfsuccAfcUfGfcaugAfcGfaagggsusg | 517 | CACCCUUCGUCAUGCAGUGGAGG | 652 |
| AD-1686543 | csusgagcAfgUfGfGfcagcagaaguL96 | 383 | asCfsuucUfgCfUfgccaCfuGfcucagscsc | 518 | GGCUGAGCAGUGGCAGCAGAAGG | 653 |
| AD-1686556 | csasgaagGfgCfCfGfccuccauucuL96 | 384 | asGfsaauGfgAfGfgcggCfcCfuucugscsu | 519 | AGCAGAAGGGCCGCCUCCAUUCC | 654 |
| AD-1686565 | csgsccucCfaUfUfCfcuacuaagguL96 | 385 | asCfscuuAfgUfAfggaaUfgGfaggcgsgsc | 520 | GCCGCCUCCAUUCCUACUAAGGG | 655 |
| AD-1686566 | csasgaauCfaUfUfCfcaaccgaccuL96 | 386 | asGfsgucGfgUfUfggaaUfgAfuucugsgsg | 521 | CCCAGAAUCAUUCCAACCGACCC | 656 |
| AD-1686576 | cscsaaccGfaCfCfCfacugcaaaguL96 | 387 | asCfsuuuGfcAfGfuggUfcGfguuggsasa | 522 | UUCCAACCGACCCACUGCAAAGA | 657 |
| AD-1686585 | cscsacugCfaAfAfGfacuaugacauL96 | 388 | asUfsgucAfuAfGfucuuUfgCfaguggsgsu | 523 | ACCCACUGCAAAGACUAUGACAG | 658 |
| AD-1686597 | csusaugaCfaGfCfAfucaaauuucuL96 | 389 | asGfsaaaUfuUfGfaugcUfgUfcauagsusc | 524 | GACUAUGACAGCAUCAAAUUUCA | 659 |
| AD-1686606 | csasucaaAfuUfUfCfaggaccugcuL96 | 390 | asGfscagGfuCfCfugaaAfuUfugaugscsu | 525 | AGCAUCAAAUUUCAGGACCUGCA | 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 | asUfsguaCfuGfUfcugcAfgGfuccugsasa | 526 | UUCAGGACCUGCAGACAGUACAG | 661 |
| AD-1686626 | asgsacagUfaCfAfGfgcuagauaauL96 | 392 | asUfsuauCfuAfGfccugUfaCfugucusgsc | 527 | GCAGACAGUACAGGCUAGAUAAC | 662 |
| AD-1686639 | usasgauaAfcCfCfAfcccaauuucuL96 | 393 | asGfsaaaUfuGfGfguggGfuUfaucuasgsc | 528 | GCUAGAUAACCCACCCAAUUUCC | 663 |
| AD-1686649 | asgsaaccUfuUfCfAfgcauaacgcuL96 | 394 | asGfscguUfaUfGfcugaAfaGfguucusgsu | 529 | ACAGAACCUUUCAGCAUAACGCC | 664 |
| AD-1686658 | csasgcauAfaCfGfCfcucacauccuL96 | 395 | asGfsgauGfuGfAfggcgUfuAfugcugsasa | 530 | UUCAGCAUAACGCCUCACAUCCC | 665 |
| AD-1686669 | csuscacaUfcCfCfAfagucuauacuL96 | 396 | asGfsuauAfgAfCfuuggGfaUfgugagsgsc | 531 | GCCUCACAUCCCAAGUCUAUACC | 666 |
| AD-1686680 | asgsucuaUfaCfCfCfuuaccugaauL96 | 397 | asUfsucaGfgUfAfaggUfaUfagacususg | 532 | CAAGUCUAUACCCUUACCUGAAG | 667 |
| AD-1686693 | ascscugaAfgAfAfUfgcuguucuuuL96 | 398 | asAfsagaAfcAfGfcauuCfuUfcaggusasa | 533 | UUACCUGAAGAAUGCUGUUCUUU | 668 |
| AD-1686709 | uscsuuucCfuAfGfCfcaccuuucuuL96 | 399 | asAfsgaaAfgGfUfggcuAfgGfaaagasasc | 534 | GUUCUUUCCUAGCCACCUUUCUG | 669 |
| AD-1686720 | csasccuuUfcUfGfGfccucccacuuL96 | 400 | asAfsgugGfgAfGfgccaGfaAfaggugsgsc | 535 | GCCACCUUUCUGGCCUCCCACUU | 670 |
| AD-1686729 | gsgsccucCfcAfCfCfUfugccugaauL96 | 401 | asUfsucaGfgGfCfaaguGfgGfaggccsasg | 536 | CUGGCCUCCCACUUGCCCUGAAA | 671 |
| AD-1686738 | csusugccCfuGfAfAfaggccaagauL96 | 402 | asUfscuuGfgCfCfuuucAfgGfgcaagsusg | 537 | CACUUGCCCUGAAAGGCCAAGAU | 672 |
| AD-1686747 | asasaggcCfaAfGfAfucaagauguuL96 | 403 | asAfscauCfuUfGfaucuUfgGfccuuuscsa | 538 | UGAAAGGCCAAGAUCAAGAUGUC | 673 |
| AD-1686751 | asgsgcauCfuUfGfAfucccagccuuL96 | 404 | asAfsggcUfgGfGfaucaAfgAfugccusgsg | 539 | CCAGGCAUCUUGAUCCCAGCCUG | 674 |
| AD-1686761 | asuscccaGfcCfUfGfacugcugcuuL96 | 405 | asAfsgcaGfcAfGfucagGfcUfgggauscsa | 540 | UGAUCCCAGCCUGACUGCUGCUA | 675 |
| AD-1686770 | usgsacugCfuGfCfUfacaucuaauuL96 | 406 | asAfsuuaGfaUfGfuagcAfgCfagucasgsg | 541 | CCUGACUGCUGCUACAUCUAAUC | 676 |
| AD-1686781 | gscscuccUfgUfCfCfcuaaacuccuL96 | 407 | asGfsgagUfuUfAfgggaCfaGfgaggcsasu | 542 | AUGCCUCCUGUCCCUAAACUCCC | 677 |
| AD-1686783 | asgscauaCfuGfAfUfgacagcccuuL96 | 408 | asAfsgggCfuGfUfcaucAfgUfaugcusgsg | 543 | CCAGCAUACUGAUGACAGCCCUC | 678 |
| AD-1686794 | gsascagcCfcUfCfUfcugacuuuauL96 | 409 | asUfsaaaGfuCfAfgagaGfgGfcugucsasu | 544 | AUGACAGCCCUCUCUGACUUUAC | 679 |
| AD-1686803 | csuscugaCfuUfUfAfccuugagauuL96 | 410 | asAfsucuCfaAfGfguaaAfgUfcagagsasg | 545 | CUCUCUGACUUUACCUUGAGAUC | 680 |
| AD-1686813 | ascscuugAfgAfUfCfugucuucauuL96 | 411 | asAfsugaAfgAfCfagauCfuCfaaggusasa | 546 | UUACCUUGAGAUCUGUCUUCAUA | 681 |

TABLE 5. Unmodified Sense and Antisense Strand Sequences of Human CIDEB 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 | GUCCUGGGCUGAGAUCCCAGU | 682 | 1269-1289 | ACUGGGAUCUCAGCCCAGGACAA | 1130 | 1267-1289 |
| AD-1699867.1 | CUGGGCUGAGAUCCCAGGUUU | 683 | 1272-1292 | AAACCUGGGAUCUCAGCCCAGGA | 1131 | 1270-1292 |
| AD-1699868.1 | UGGGCUGAGAUCCCAGGUUUU | 684 | 1273-1293 | AAAACCTGGGATCUCAGCCCAGG | 1132 | 1271-1293 |
| AD-1699869.1 | GGGCUGAGAUCCCAGGUUUGU | 685 | 1274-1294 | ACAAACCUGGGAUCUCAGCCCAG | 1133 | 1272-1294 |
| AD-1699870.1 | GGCUGAGAUCCCAGGUUUGUU | 686 | 1275-1295 | AACAAACCUGGGAUCUCAGCCCA | 1134 | 1273-1295 |
| AD-1699871.1 | GCUGAGAUCCCAGGUUUGUAU | 687 | 1276-1296 | ATACAAACCUGGGAUCUCAGCCC | 1135 | 1274-1296 |
| AD-1699872.1 | CUGAGAUCCCAGGUUUGUAAU | 688 | 1277-1297 | ATUACAAACCUGGGAUCUCAGCC | 1136 | 1275-1297 |
| AD-1699873.1 | UGAGAUCCCAGGUUUGUAACU | 689 | 1278-1298 | AGUUACAAACCTGGGAUCUCAGC | 1137 | 1276-1298 |
| AD-1699875.1 | AGAUCCCAGGUUUGUAACAGU | 690 | 1280-1300 | ACUGTUACAAACCUGGGAUCUCA | 1138 | 1278-1300 |
| AD-1699882.1 | AGGUUUGUAACAGAAAACACU | 691 | 1287-1307 | AGUGTUTUCUGTUACAAACCUGG | 1139 | 1285-1307 |
| AD-1699891.1 | ACAGAAAACACCACUAAAGCU | 692 | 1296-1316 | AGCUTUAGUGGTGUUUUCUGUUA | 1140 | 1294-1316 |
| AD-1699892.1 | CAGAAAACACCACUAAAGCCU | 693 | 1297-1317 | AGGCTUTAGUGGUGUUUUCUGUU | 1141 | 1295-1317 |
| AD-1699904.1 | AGAACCACCCAGCCCAGAAGU | 694 | 1329-1349 | ACUUCUGGGCUGGGUGGUUCUCU | 1142 | 1327-1349 |
| AD-1699907.1 | ACCACCCAGCCCAGAAGUUCU | 695 | 1332-1352 | AGAACUTCUGGGCUGGGUGGUUC | 1143 | 1330-1352 |
| AD-1699948.1 | CACCAUGGAGUACCUCUCAGU | 696 | 1373-1393 | ACUGAGAGGUACUCCAUGGUGGA | 1144 | 1371-1393 |
| AD-1699949.1 | ACCAUGGAGUACCUCUCAGCU | 697 | 1374-1394 | AGCUGAGAGGUACUCCAUGGUGG | 1145 | 1372-1394 |
| AD-1699951.1 | CAUGGAGUACCUCUCAGCUCU | 698 | 1376-1396 | AGAGCUGAGAGGUACUCCAUGGU | 1146 | 1374-1396 |
| AD-1699964.1 | CUUACUCAGGUCAGUAUCUAU | 699 | 1409-1429 | ATAGAUACUGACCUGAGUAAGUC | 1147 | 1407-1429 |
| AD-1699967.1 | ACUCAGGUCAGUAUCUAAUAU | 700 | 1412-1432 | ATAUTAGAUACTGACCUGAGUAA | 1148 | 1410-1432 |
| AD-1699970.1 | CAGGUCAGUAUCUAAUAUAAU | 701 | 1415-1435 | ATUATATUAGATACUGACCUGAG | 1149 | 1413-1435 |
| AD-1699971.1 | AGGUCAGUAUCUAAUAUAAGU | 702 | 1416-1436 | ACUUAUAUUAGAUACUGACCUGA | 1150 | 1414-1436 |
| AD-1699972.1 | GGUCAGUAUCUAAUAUAAGCU | 703 | 1417-1437 | AGCUTATAUUAGAUACUGACCUG | 1151 | 1415-1437 |
| AD-1699973.1 | GUCAGUAUCUAAUAUAAGCUU | 704 | 1418-1438 | AAGCTUAUAUUAGAUACUGACCU | 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 | AGUAUCUAAUAUAAGCUCGGU | 705 | 1421-1441 | ACCGAGCUUAUAUUAGAUACUGA | 1153 | 1419-1441 |
| AD-1699977.1 | GUAUCUAAUAUAAGCUCGGAU | 706 | 1422-1442 | ATCCGAGCUUAUAUUAGAUACUG | 1154 | 1420-1442 |
| AD-1699978.1 | UAUCUAAUAUAAGCUCGGAGU | 707 | 1423-1443 | ACUCCGAGCUUAUAUUAGAUACU | 1155 | 1421-1443 |
| AD-1699979.1 | AUCUAAUAUAAGCUCGGAGUU | 708 | 1424-1444 | AACUCCGAGCUUAUAUUAGAUAC | 1156 | 1422-1444 |
| AD-1699982.1 | UAAUAUAAGCUCGGAGUUUGU | 709 | 1427-1447 | ACAAACTCCGAGCUUAUAUUAGA | 1157 | 1425-1447 |
| AD-1699983.1 | AAUAUAAGCUCGGAGUUUGGU | 710 | 1428-1448 | ACCAAACUCCGAGCUUAUAUUAG | 1158 | 1426-1448 |
| AD-1699984.1 | AUAUAAGCUCGGAGUUUGGAU | 711 | 1429-1449 | ATCCAAACUCCGAGCUUAUAUUA | 1159 | 1427-1449 |
| AD-1699985.1 | UAUAAGCUCGGAGUUUGGACU | 712 | 1430-1450 | AGUCCAAACUCCGAGCUUAUAUU | 1160 | 1428-1450 |
| AD-1699986.1 | AUAAGCUCGGAGUUUGGACGU | 713 | 1431-1451 | ACGUCCAAACUCCGAGCUUAUAU | 1161 | 1429-1451 |
| AD-1699987.1 | UAAGCUCGGAGUUUGGACGGU | 714 | 1432-1452 | ACCGTCCAAACTCCGAGCUUAUA | 1162 | 1430-1452 |
| AD-1699988.1 | AAGCUCGGAGUUUGGACGGAU | 715 | 1433-1453 | ATCCGUCCAAACUCCGAGCUUAU | 1163 | 1431-1453 |
| AD-1699989.1 | AGCUCGGAGUUUGGACGGAGU | 716 | 1434-1454 | ACUCCGTCCAAACUCCGAGCUUA | 1164 | 1432-1454 |
| AD-1699990.1 | GCUCGGAGUUUGGACGGAGGU | 717 | 1435-1455 | ACCUCCGUCCAAACUCCGAGCUU | 1165 | 1433-1455 |
| AD-1700015.1 | AGCGACCUUUCCGUGUCUGUU | 718 | 1480-1500 | AACAGACACGGAAAGGUCGCUGG | 1166 | 1478-1500 |
| AD-1700035.1 | GAUCACAAGCGGACCAUCCGU | 719 | 1500-1520 | ACGGAUGGUCCGCUUGUGAUCAC | 1167 | 1498-1520 |
| AD-1700037.1 | UCACAAGCGGACCAUCCGGAU | 720 | 1502-1522 | ATCCGGAUGGUCCGCUUGUGAUC | 1168 | 1500-1522 |
| AD-1700038.1 | CACAAGCGGACCAUCCGGAAU | 721 | 1503-1523 | ATUCCGGAUGGTCCGCUUGUGAU | 1169 | 1501-1523 |
| AD-1700039.1 | ACAAGCGGACCAUCCGGAAAU | 722 | 1504-1524 | ATUUCCGGAUGGUCCGCUUGUGA | 1170 | 1502-1524 |
| AD-1700040.1 | CAAGCGGACCAUCCGGAAAGU | 723 | 1505-1525 | ACUUTCCGGAUGGUCCGCUUGUG | 1171 | 1503-1525 |
| AD-1700041.1 | AAGCGGACCAUCCGGAAAGGU | 724 | 1506-1526 | ACCUTUCCGGATGGUCCGCUUGU | 1172 | 1504-1526 |
| AD-1700077.1 | GGAGCUGCUAGCCAAAGCAUU | 725 | 1547-1567 | AAUGCUTUGGCTAGCAGCUCCUG | 1173 | 1545-1567 |
| AD-1700080.1 | GCUGCUAGCCAAAGCAUUGGU | 726 | 1550-1570 | ACCAAUGCUUUGGCUAGCAGCUC | 1174 | 1548-1570 |
| AD-1700081.1 | CUGCUAGCCAAAGCAUUGGAU | 727 | 1551-1571 | ATCCAATGCUUTGGCUAGCAGCU | 1175 | 1549-1571 |
| AD-1700082.1 | UGCUAGCCAAAGCAUUGGAGU | 728 | 1552-1572 | ACUCCAAUGCUTUGGCUAGCAGC | 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 | ACAGTAGGGUCTC 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 GCACUCCAUU | 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 UGCAGUUCCA | 1195 | 1616-1638 |
| AD-1700149.1 | AACUGCAGUGG ACAGUGAGGU | 748 | 1619-1639 | ACCUCACUGUCCA CUGCAGUUCC | 1196 | 1617-1639 |
| AD-1700156.1 | GUGGACAGUGA GGACUUCUUU | 749 | 1626-1646 | AAAGAAGUCCUCA CUGUCCACUG | 1197 | 1624-1646 |
| AD-1700158.1 | GGACAGUGAGG ACUUCUUCCU | 750 | 1628-1648 | AGGAAGAAGUCC UCACUGUCCAC | 1198 | 1626-1648 |
| AD-1700159.1 | GACAGUGAGGA CUUCUUCCAU | 751 | 1629-1649 | ATGGAAGAAGUCC UCACUGUCCA | 1199 | 1627-1649 |
| AD-1700160.1 | ACAGUGAGGAC UUCUUCCAGU | 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 | AGGACUUCUUCCAGCUGCUGU | 753 | 1636-1656 | ACAGCAGCUGGAAGAAGUCCUCA | 1201 | 1634-1656 |
| AD-1700167.1 | GGACUUCUUCCAGCUGCUGGU | 754 | 1637-1657 | ACCAGCAGCUGGAAGAAGUCCUC | 1202 | 1635-1657 |
| AD-1700180.1 | CUGCUGGAGGAUGACACGUGU | 755 | 1650-1670 | ACACGUGUCAUCCUCCAGCAGCU | 1203 | 1648-1670 |
| AD-1700187.1 | AGGAUGACACGUGCCUGAUGU | 756 | 1657-1677 | ACAUCAGGCACGUGUCAUCCUCC | 1204 | 1655-1677 |
| AD-1700188.1 | GGAUGACACGUGCCUGAUGGU | 757 | 1658-1678 | ACCATCAGGCACGUGUCAUCCUC | 1205 | 1656-1678 |
| AD-1700189.1 | GAUGACACGUGCCUGAUGGUU | 758 | 1659-1679 | AACCAUCAGGCACGUGUCAUCCU | 1206 | 1657-1679 |
| AD-1700190.1 | AUGACACGUGCCUGAUGGUGU | 759 | 1660-1680 | ACACCATCAGGCACGUGUCAUCC | 1207 | 1658-1680 |
| AD-1700191.1 | UGACACGUGCCUGAUGGUGUU | 760 | 1661-1681 | AACACCAUCAGGCACGUGUCAUC | 1208 | 1659-1681 |
| AD-1700193.1 | ACACGUGCCUGAUGGUGUUGU | 761 | 1663-1683 | ACAACACCAUCAGGCACGUGUCA | 1209 | 1661-1683 |
| AD-1700213.1 | CAGUCUGGUCAGAGCUGGAGU | 762 | 1683-1703 | ACUCCAGCUCUGACCAGACUGCA | 1210 | 1681-1703 |
| AD-1700242.1 | GAGUGGAGUGCUGUCAUAUGU | 763 | 1712-1732 | ACAUAUGACAGCACUCCACUCCU | 1211 | 1710-1732 |
| AD-1700243.1 | AGUGGAGUGCUGUCAUAUGGU | 764 | 1713-1733 | ACCATATGACAGCACUCCACUCC | 1212 | 1711-1733 |
| AD-1700244.1 | GUGGAGUGCUGUCAUAUGGCU | 765 | 1714-1734 | AGCCAUAUGACAGCACUCCACUC | 1213 | 1712-1734 |
| AD-1700245.1 | UGGAGUGCUGUCAUAUGGCCU | 766 | 1715-1735 | AGGCCATAUGACAGCACUCCACU | 1214 | 1713-1735 |
| AD-1700248.1 | AGUGCUGUCAUAUGGCCUGGU | 767 | 1718-1738 | ACCAGGCCAUATGACAGCACUCC | 1215 | 1716-1738 |
| AD-1700249.1 | GUGCUGUCAUAUGGCCUGGGU | 768 | 1719-1739 | ACCCAGGCCAUAUGACAGCACUC | 1216 | 1717-1739 |
| AD-1700250.1 | UGCUGUCAUAUGGCCUGGGAU | 769 | 1720-1740 | ATCCCAGGCCATAUGACAGCACU | 1217 | 1718-1740 |
| AD-1700252.1 | CUGUCAUAUGGCCUGGGACGU | 770 | 1722-1742 | ACGUCCCAGGCCAUAUGACAGCA | 1218 | 1720-1742 |
| AD-1700272.1 | AGGCCCAAGCACAGCAAGGAU | 771 | 1746-1766 | ATCCTUGCUGUGCUUGGGCCUCU | 1219 | 1744-1766 |
| AD-1700279.1 | AGCACAGCAAGGACAUCGCCU | 772 | 1753-1773 | AGGCGATGUCCTUGCUGUGCUUG | 1220 | 1751-1773 |
| AD-1700280.1 | GCACAGCAAGGACAUCGCCCU | 773 | 1754-1774 | AGGGCGAUGUCCUUGCUGUGCUU | 1221 | 1752-1774 |
| AD-1700303.1 | UCACCUUUGACGUGUACAAGU | 774 | 1777-1797 | ACUUGUACACGTCAAAGGUGAAU | 1222 | 1775-1797 |
| AD-1700309.1 | UUGACGUGUACAAGCAAAACU | 775 | 1783-1803 | AGUUTUGCUUGTACACGUCAAAG | 1223 | 1781-1803 |
| AD-1700312.1 | ACGUGUACAAGCAAAACCCUU | 776 | 1786-1806 | AAGGGUTUUGCTUGUACACGUCA | 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 | GUGUACAAGCAAAACCCUCGU | 777 | 1788-1808 | ACGAGGGUUUUGCUUGUACACGU | 1225 | 1786-1808 |
| AD-1700315.1 | UGUACAAGCAAAACCCUCGAU | 778 | 1789-1809 | ATCGAGGGUUUTGCUUGUACACG | 1226 | 1787-1809 |
| AD-1700316.1 | GUACAAGCAAAACCCUCGAGU | 779 | 1790-1810 | ACUCGAGGGUUTUGCUUGUACAC | 1227 | 1788-1810 |
| AD-1700317.1 | UACAAGCAAAACCCUCGAGAU | 780 | 1791-1811 | ATCUCGAGGGUTUUGCUUGUACA | 1228 | 1789-1811 |
| AD-1700318.1 | ACAAGCAAAACCCUCGAGACU | 781 | 1792-1812 | AGUCTCGAGGGTUUUGCUUGUAC | 1229 | 1790-1812 |
| AD-1700323.1 | CAAAACCCUCGAGACCUCUUU | 782 | 1797-1817 | AAAGAGGUCUCGAGGGUUUUGCU | 1230 | 1795-1817 |
| AD-1700324.1 | AAAACCCUCGAGACCUCUUUU | 783 | 1798-1818 | AAAAGAGGUCUCGAGGGUUUUGC | 1231 | 1796-1818 |
| AD-1700325.1 | AAACCCUCGAGACCUCUUUGU | 784 | 1799-1819 | ACAAAGAGGUCTCGAGGGUUUUG | 1232 | 1797-1819 |
| AD-1700327.1 | ACCCUCGAGACCUCUUUGGCU | 785 | 1801-1821 | AGCCAAAGAGGTCUCGAGGGUUU | 1233 | 1799-1821 |
| AD-1700328.1 | CCCUCGAGACCUCUUUGGCAU | 786 | 1802-1822 | ATGCCAAAGAGGUCUCGAGGGUU | 1234 | 1800-1822 |
| AD-1700329.1 | CCUCGAGACCUCUUUGGCAGU | 787 | 1803-1823 | ACUGCCAAAGAGGUCUCGAGGGU | 1235 | 1801-1823 |
| AD-1700336.1 | ACCUCUUUGGCAGCCUGAAUU | 788 | 1810-1830 | AAUUCAGGCUGCCAAAGAGGUCU | 1236 | 1808-1830 |
| AD-1700339.1 | UCUUUGGCAGCCUGAAUGUCU | 789 | 1813-1833 | AGACAUTCAGGCUGCCAAAGAGG | 1237 | 1811-1833 |
| AD-1700344.1 | GGCAGCCUGAAUGUCAAAGCU | 790 | 1818-1838 | AGCUTUGACAUTCAGGCUGCCAA | 1238 | 1816-1838 |
| AD-1700350.1 | CUGAAUGUCAAAGCCACAUUU | 791 | 1824-1844 | AAAUGUGGCUUTGACAUUCAGGC | 1239 | 1822-1844 |
| AD-1700352.1 | GAAUGUCAAAGCCACAUUCUU | 792 | 1826-1846 | AAGAAUGUGGCTUUGACAUUCAG | 1240 | 1824-1846 |
| AD-1700353.1 | AAUGUCAAAGCCACAUUCUAU | 793 | 1827-1847 | ATAGAATGUGGCUUUGACAUUCA | 1241 | 1825-1847 |
| AD-1700354.1 | AUGUCAAAGCCACAUUCUACU | 794 | 1828-1848 | AGUAGAAUGUGGCUUUGACAUUC | 1242 | 1826-1848 |
| AD-1700355.1 | UGUCAAAGCCACAUUCUACGU | 795 | 1829-1849 | ACGUAGAAUGUGGCUUUGACAUU | 1243 | 1827-1849 |
| AD-1700356.1 | GUCAAAGCCACAUUCUACGGU | 796 | 1830-1850 | ACCGTAGAAUGTGGCUUUGACAU | 1244 | 1828-1850 |
| AD-1700357.1 | UCAAAGCCACAUUCUACGGGU | 797 | 1831-1851 | ACCCGUAGAAUGUGGCUUUGACA | 1245 | 1829-1851 |
| AD-1700358.1 | CAAAGCCACAUUCUACGGGCU | 798 | 1832-1852 | AGCCCGTAGAATGUGGCUUUGAC | 1246 | 1830-1852 |
| AD-1700359.1 | AAAGCCACAUUCUACGGGCUU | 799 | 1833-1853 | AAGCCCGUAGAAUGUGGCUUUGA | 1247 | 1831-1853 |
| AD-1700365.1 | ACAUUCUACGGGCUCUACUCU | 800 | 1839-1859 | AGAGTAGAGCCCGUAGAAUGUGG | 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 | CAUUCUACGGGCUCUACUCUU | 801 | 1840-1860 | AAGAGUAGAGCCCGUAGAAUGUG | 1249 | 1838-1860 |
| AD-1700368.1 | UUCUACGGGCUCUACUCUAUU | 802 | 1842-1862 | AAUAGAGUAGAGCCCGUAGAAUG | 1250 | 1840-1862 |
| AD-1700369.1 | UCUACGGGCUCUACUCUAUGU | 803 | 1843-1863 | ACAUAGAGUAGAGCCCGUAGAAU | 1251 | 1841-1863 |
| AD-1700370.1 | CUACGGGCUCUACUCUAUGAU | 804 | 1844-1864 | ATCATAGAGUAGAGCCCGUAGAA | 1252 | 1842-1864 |
| AD-1700371.1 | UACGGGCUCUACUCUAUGAGU | 805 | 1845-1865 | ACUCAUAGAGUAGAGCCCGUAGA | 1253 | 1843-1865 |
| AD-1700372.1 | ACGGGCUCUACUCUAUGAGUU | 806 | 1846-1866 | AACUCATAGAGTAGAGCCCGUAG | 1254 | 1844-1866 |
| AD-1700374.1 | GGGCUCUACUCUAUGAGUUGU | 807 | 1848-1868 | ACAACUCAUAGAGUAGAGCCCGU | 1255 | 1846-1868 |
| AD-1700375.1 | GGCUCUACUCUAUGAGUUGUU | 808 | 1849-1869 | AACAACTCAUAGAGUAGAGCCCG | 1256 | 1847-1869 |
| AD-1700376.1 | GCUCUACUCUAUGAGUUGUGU | 809 | 1850-1870 | ACACAACUCAUAGAGUAGAGCCC | 1257 | 1848-1870 |
| AD-1700378.1 | UCUACUCUAUGAGUUGUGACU | 810 | 1852-1872 | AGUCACAACUCAUAGAGUAGAGC | 1258 | 1850-1872 |
| AD-1700383.1 | UCUAUGAGUUGUGACUUUCAU | 811 | 1857-1877 | ATGAAAGUCACAACUCAUAGAGU | 1259 | 1855-1877 |
| AD-1700384.1 | CUAUGAGUUGUGACUUUCAAU | 812 | 1858-1878 | ATUGAAAGUCACAACUCAUAGAG | 1260 | 1856-1878 |
| AD-1700385.1 | UAUGAGUUGUGACUUUCAAGU | 813 | 1859-1879 | ACUUGAAAGUCACAACUCAUAGA | 1261 | 1857-1879 |
| AD-1700386.1 | AUGAGUUGUGACUUUCAAGGU | 814 | 1860-1880 | ACCUTGAAAGUCACAACUCAUAG | 1262 | 1858-1880 |
| AD-1700387.1 | UGAGUUGUGACUUUCAAGGAU | 815 | 1861-1881 | ATCCTUGAAAGTCACAACUCAUA | 1263 | 1859-1881 |
| AD-1700388.1 | GAGUUGUGACUUUCAAGGACU | 816 | 1862-1882 | AGUCCUTGAAAGUCACAACUCAU | 1264 | 1860-1882 |
| AD-1700408.1 | UUGGCCCAAAGAAAGUACUCU | 817 | 1882-1902 | AGAGTACUUUCTUUGGGCCAAGU | 1265 | 1880-1902 |
| AD-1700410.1 | GGCCCAAAGAAAGUACUCAGU | 818 | 1884-1904 | ACUGAGTACUUTCUUUGGGCCAA | 1266 | 1882-1904 |
| AD-1700411.1 | GCCCAAAGAAAGUACUCAGGU | 819 | 1885-1905 | ACCUGAGUACUTUCUUUGGGCCA | 1267 | 1883-1905 |
| AD-1700413.1 | CCAAAGAAAGUACUCAGGGAU | 820 | 1887-1907 | ATCCCUGAGUACUUUCUUUGGGC | 1268 | 1885-1907 |
| AD-1700414.1 | CAAAGAAAGUACUCAGGGAGU | 821 | 1888-1908 | ACUCCCTGAGUACUUUCUUUGGG | 1269 | 1886-1908 |
| AD-1700422.1 | GUACUCAGGGAGCUCCUUCGU | 822 | 1896-1916 | ACGAAGGAGCUCCCUGAGUACUU | 1270 | 1894-1916 |
| AD-1700423.1 | UACUCAGGGAGCUCCUUCGUU | 823 | 1897-1917 | AACGAAGGAGCTCCCUGAGUACU | 1271 | 1895-1917 |
| AD-1700424.1 | ACUCAGGGAGCUCCUUCGUUU | 824 | 1898-1918 | AAACGAAGGAGCUCCCUGAGUAC | 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 | CUCAGGGAGCUCCUUCGUUGU | 825 | 1899-1919 | ACAACGAAGGAGCUCCCUGAGUA | 1273 | 1897-1919 |
| AD-1700426.1 | UCAGGGAGCUCCUUCGUUGGU | 826 | 1900-1920 | ACCAACGAAGGAGCUCCCUGAGU | 1274 | 1898-1920 |
| AD-1700427.1 | CAGGGAGCUCCUUCGUUGGAU | 827 | 1901-1921 | ATCCAACGAAGGAGCUCCCUGAG | 1275 | 1899-1921 |
| AD-1700428.1 | AGGGAGCUCCUUCGUUGGACU | 828 | 1902-1922 | AGUCCAACGAAGGAGCUCCCUGA | 1276 | 1900-1922 |
| AD-1700439.1 | UCGUUGGACCUCCACACUGCU | 829 | 1913-1933 | AGCAGUGUGGAGGUCCAACGAAG | 1277 | 1911-1933 |
| AD-1700461.1 | CAAGGCCUGGGCCAUAUGUUU | 830 | 1935-1955 | AAACAUAUGGCCCAGGCCUUGCA | 1278 | 1933-1955 |
| AD-1700462.1 | AAGGCCUGGGCCAUAUGUUGU | 831 | 1936-1956 | ACAACATAUGGCCCAGGCCUUGC | 1279 | 1934-1956 |
| AD-1700464.1 | GGCCUGGGCCAUAUGUUGCUU | 832 | 1938-1958 | AAGCAACAUAUGGCCCAGGCCUU | 1280 | 1936-1958 |
| AD-1700465.1 | GCCUGGGCCAUAUGUUGCUGU | 833 | 1939-1959 | ACAGCAACAUATGGCCCAGGCCU | 1281 | 1937-1959 |
| AD-1700468.1 | UGGGCCAUAUGUUGCUGGGAU | 834 | 1942-1962 | ATCCCAGCAACAUAUGGCCCAGG | 1282 | 1940-1962 |
| AD-1700473.1 | CAUAUGUUGCUGGGAAUUUCU | 835 | 1947-1967 | AGAAAUTCCCAGCAACAUAUGGC | 1283 | 1945-1967 |
| AD-1700474.1 | AUAUGUUGCUGGGAAUUUCCU | 836 | 1948-1968 | AGGAAATUCCCAGCAACAUAUGG | 1284 | 1946-1968 |
| AD-1700476.1 | AUGUUGCUGGGAAUUUCCUCU | 837 | 1950-1970 | AGAGGAAAUUCCCAGCAACAUAU | 1285 | 1948-1970 |
| AD-1700477.1 | UGUUGCUGGGAAUUUCCUCCU | 838 | 1951-1971 | AGGAGGAAAUUCCCAGCAACAUA | 1286 | 1949-1971 |
| AD-1700479.1 | UUGCUGGGAAUUUCCUCCACU | 839 | 1953-1973 | AGUGGAGGAAATUCCCAGCAACA | 1287 | 1951-1973 |
| AD-1700482.1 | CUGGGAAUUUCCUCCACCCUU | 840 | 1956-1976 | AAGGGUGGAGGAAAUUCCCAGCA | 1288 | 1954-1976 |
| AD-1700485.1 | GGAAUUUCCUCCACCCUUCGU | 841 | 1959-1979 | ACGAAGGGUGGAGGAAAUUCCCA | 1289 | 1957-1979 |
| AD-1700486.1 | GAAUUUCCUCCACCCUUCGUU | 842 | 1960-1980 | AACGAAGGGUGGAGGAAAUUCCC | 1290 | 1958-1980 |
| AD-1700487.1 | AAUUUCCUCCACCCUUCGUCU | 843 | 1961-1981 | AGACGAAGGGUGGAGGAAAUUCC | 1291 | 1959-1981 |
| AD-1700488.1 | AUUUCCUCCACCCUUCGUCAU | 844 | 1962-1982 | ATGACGAAGGGTGGAGGAAAUUC | 1292 | 1960-1982 |
| AD-1700489.1 | UUUCCUCCACCCUUCGUCAUU | 845 | 1963-1983 | AAUGACGAAGGGUGGAGGAAAUU | 1293 | 1961-1983 |
| AD-1700490.1 | UUCCUCCACCCUUCGUCAUGU | 846 | 1964-1984 | ACAUGACGAAGGGUGGAGGAAAU | 1294 | 1962-1984 |
| AD-1700517.1 | AAGGGCCGCCUCCAUUCCUAU | 847 | 2013-2033 | ATAGGAAUGGAGGCGGCCCUUCU | 1295 | 2011-2033 |
| AD-1700519.1 | GGGCCGCCUCCAUUCCUACUU | 848 | 2015-2035 | AAGUAGGAAUGGAGGCGGCCCUU | 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 | GGCCGCCUCCAUUCCUACUAU | 849 | 2016-2036 | ATAGTAGGAAUGGAGGCGGCCCU | 1297 | 2014-2036 |
| AD-1700522.1 | CCGCCUCCAUUCCUACUAAGU | 850 | 2018-2038 | ACUUAGTAGGAAUGGAGGCGGCC | 1298 | 2016-2038 |
| AD-1700542.1 | CCCACUGCAAAGACUAUGACU | 851 | 2076-2096 | AGUCAUAGUCUTUGCAGUGGGUC | 1299 | 2074-2096 |
| AD-1700544.1 | CACUGCAAAGACUAUGACAGU | 852 | 2078-2098 | ACUGTCAUAGUCUUUGCAGUGGG | 1300 | 2076-2098 |
| AD-1700550.1 | AAAGACUAUGACAGCAUCAAU | 853 | 2084-2104 | ATUGAUGCUGUCAUAGUCUUUGC | 1301 | 2082-2104 |
| AD-1700553.1 | GACUAUGACAGCAUCAAAUUU | 854 | 2087-2107 | AAAUTUGAUGCTGUCAUAGUCUU | 1302 | 2085-2107 |
| AD-1700554.1 | ACUAUGACAGCAUCAAAUUUU | 855 | 2088-2108 | AAAATUTGAUGCUGUCAUAGUCU | 1303 | 2086-2108 |
| AD-1700555.1 | CUAUGACAGCAUCAAAUUUCU | 856 | 2089-2109 | AGAAAUTUGAUGCUGUCAUAGUC | 1304 | 2087-2109 |
| AD-1700556.1 | UAUGACAGCAUCAAAUUUCAU | 857 | 2090-2110 | ATGAAATUUGATGCUGUCAUAGU | 1305 | 2088-2110 |
| AD-1700557.1 | AUGACAGCAUCAAAUUUCAGU | 858 | 2091-2111 | ACUGAAAUUUGAUGCUGUCAUAG | 1306 | 2089-2111 |
| AD-1700558.1 | UGACAGCAUCAAAUUUCAGGU | 859 | 2092-2112 | ACCUGAAAUUUGAUGCUGUCAUA | 1307 | 2090-2112 |
| AD-1700560.1 | ACAGCAUCAAAUUUCAGGACU | 860 | 2094-2114 | AGUCCUGAAAUTUGAUGCUGUCA | 1308 | 2092-2114 |
| AD-1700563.1 | GCAUCAAAUUUCAGGACCUGU | 861 | 2097-2117 | ACAGGUCCUGAAAUUUGAUGCUG | 1309 | 2095-2117 |
| AD-1700566.1 | UCAAAUUUCAGGACCUGCAGU | 862 | 2100-2120 | ACUGCAGGUCCTGAAAUUUGAUG | 1310 | 2098-2120 |
| AD-1700573.1 | UCAGGACCUGCAGACAGUACU | 863 | 2107-2127 | AGUACUGUCUGCAGGUCCUGAAA | 1311 | 2105-2127 |
| AD-1700575.1 | AGGACCUGCAGACAGUACAGU | 864 | 2109-2129 | ACUGTACUGUCTGCAGGUCCUGA | 1312 | 2107-2129 |
| AD-1700576.1 | GGACCUGCAGACAGUACAGGU | 865 | 2110-2130 | ACCUGUACUGUCUGCAGGUCCUG | 1313 | 2108-2130 |
| AD-1700578.1 | ACCUGCAGACAGUACAGGCUU | 866 | 2112-2132 | AAGCCUGUACUGUCUGCAGGUCC | 1314 | 2110-2132 |
| AD-1700580.1 | CUGCAGACAGUACAGGCUAGU | 867 | 2114-2134 | ACUAGCCUGUACUGUCUGCAGGU | 1315 | 2112-2134 |
| AD-1700582.1 | GCAGACAGUACAGGCUAGAUU | 868 | 2116-2136 | AAUCTAGCCUGTACUGUCUGCAG | 1316 | 2114-2136 |
| AD-1700623.1 | ACGCCUCACAUCCCAAGUCUU | 869 | 2194-2214 | AAGACUTGGGATGUGAGGCGUUA | 1317 | 2192-2214 |
| AD-1700670.1 | UUCCUAGCCACCUUUCUGGCU | 870 | 2241-2261 | AGCCAGAAAGGTGGCUAGGAAAG | 1318 | 2239-2261 |
| AD-1700671.1 | UCCUAGCCACCUUUCUGGCCU | 871 | 2242-2262 | AGGCCAGAAAGGUGGCUAGGAAA | 1319 | 2240-2262 |
| AD-1700680.1 | CCUUUCUGGCCUCCCACUUGU | 872 | 2251-2271 | ACAAGUGGGAGGCCAGAAAGGUG | 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 | CUUUCUGGCCUCCCACUUGCU | 873 | 2252-2272 | AGCAAGTGGGAGGCCAGAAAGGU | 1321 | 2250-2272 |
| AD-1700684.1 | UCUGGCCUCCCACUUGCCCUU | 874 | 2255-2275 | AAGGGCAAGUGGGAGGCCAGAAA | 1322 | 2253-2275 |
| AD-1700710.1 | GGCAUCUUGAUCCCAGCCUGU | 875 | 2302-2322 | ACAGGCTGGGATCAAGAUGCCUG | 1323 | 2300-2322 |
| AD-1700736.1 | AAUGCCUCCUGUCCCUAAACU | 876 | 2348-2368 | AGUUTAGGGACAGGAGGCAUUGG | 1324 | 2346-2368 |
| AD-1700737.1 | AUGCCUCCUGUCCCUAAACUU | 877 | 2349-2369 | AAGUTUAGGGACAGGAGGCAUUG | 1325 | 2347-2369 |
| AD-1700738.1 | UGCCUCCUGUCCCUAAACUCU | 878 | 2350-2370 | AGAGTUTAGGGACAGGAGGCAUU | 1326 | 2348-2370 |
| AD-1700780.1 | CAAACUAACAAAAACAUUUCU | 879 | 2434-2454 | AGAAAUGUUUUTGUUAGUUUGAG | 1327 | 2432-2454 |
| AD-1700781.1 | AAACUAACAAAAACAUUUCCU | 880 | 2435-2455 | AGGAAATGUUUTUGUUAGUUUGA | 1328 | 2433-2455 |
| AD-1700782.1 | AACUAACAAAAACAUUUCCAU | 881 | 2436-2456 | ATGGAAAUGUUTUUGUUAGUUUG | 1329 | 2434-2456 |
| AD-1700791.1 | GUCCUGGGCUGAGAUCCCAGU | 882 | 1269-1289 | ACUGGGAUCUCAGCCCAGGACAA | 1330 | 1267-1289 |
| AD-1700793.1 | UGAGAUCCCAGGUUUGUAACU | 883 | 1278-1298 | AGUUACAAACCUGGGAUCUCAGC | 1331 | 1276-1298 |
| AD-1700794.1 | GAGAUCCCAGGUUUGUAACAU | 884 | 1279-1299 | AUGUTACAAACCUGGGAUCUCAG | 1332 | 1277-1299 |
| AD-1700795.1 | GAUCCCAGGUUUGUAACAGAU | 885 | 1281-1301 | AUCUGUTACAAACCUGGGAUCUC | 1333 | 1279-1301 |
| AD-1700796.1 | CCAGGUUUGUAACAGAAAACU | 886 | 1285-1305 | AGUUTUCUGUUACAAACCUGGGA | 1334 | 1283-1305 |
| AD-1700797.1 | CAGGUUUGUAACAGAAAACAU | 887 | 1286-1306 | AUGUTUTCUGUUACAAACCUGGG | 1335 | 1284-1306 |
| AD-1700798.1 | AGGUUUGUAACAGAAAACACU | 888 | 1287-1307 | AGUGTUTUCUGUUACAAACCUGG | 1336 | 1285-1307 |
| AD-1700799.1 | GGUUUGUAACAGAAAACACCU | 889 | 1288-1308 | AGGUGUTUUCUGUUACAAACCUG | 1337 | 1286-1308 |
| AD-1700800.1 | UAACAGAAAACACCACUAAAU | 890 | 1294-1314 | AUUUAGTGGUGUUUUCUGUUACA | 1338 | 1292-1314 |
| AD-1700801.1 | CAGAAAACACCACUAAAGCCU | 891 | 1297-1317 | AGGCTUTAGUGGUGUUUUCUGUU | 1339 | 1295-1317 |
| AD-1700802.1 | CACAGGAGAGAACCACCCAGU | 892 | 1321-1341 | ACUGGGTGGUUCUCUCCUGUGCU | 1340 | 1319-1341 |
| AD-1700804.1 | GAACCACCCAGCCCAGAAGUU | 893 | 1330-1350 | AACUTCTGGGCUGGGUGGUUCUC | 1341 | 1328-1350 |
| AD-1700805.1 | AACCACCCAGCCCAGAAGUUU | 894 | 1331-1351 | AAACTUCUGGGCUGGGUGGUUCU | 1342 | 1329-1351 |
| AD-1700806.1 | ACCACCCAGCCCAGAAGUUCU | 895 | 1332-1352 | AGAACUTCUGGGCUGGGUGGUUC | 1343 | 1330-1352 |
| AD-1700807.1 | CCACCCAGCCCAGAAGUUCCU | 896 | 1333-1353 | AGGAACTUCUGGGCUGGGUGGUU | 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 | CACCCAGCCCAGAAGUUCCAU | 897 | 1334-1354 | AUGGAACUUCUGGGCUGGGUGGU | 1345 | 1332-1354 |
| AD-1700809.1 | CCAGAAGUUCCAGGGAAGGAU | 898 | 1342-1362 | AUCCTUCCCUGGAACUUCUGGGC | 1346 | 1340-1362 |
| AD-1700810.1 | CAGAAGUUCCAGGGAAGGAAU | 899 | 1343-1363 | AUUCCUTCCCUGGAACUUCUGGG | 1347 | 1341-1363 |
| AD-1700811.1 | AGAAGUUCCAGGGAAGGAACU | 900 | 1344-1364 | AGUUCCTUCCCUGGAACUUCUGG | 1348 | 1342-1364 |
| AD-1700812.1 | GAAGUUCCAGGGAAGGAACUU | 901 | 1345-1365 | AAGUTCCUUCCCUGGAACUUCUG | 1349 | 1343-1365 |
| AD-1700813.1 | AAGUUCCAGGGAAGGAACUCU | 902 | 1346-1366 | AGAGTUCCUUCCCUGGAACUUCU | 1350 | 1344-1366 |
| AD-1700814.1 | AGUUCCAGGGAAGGAACUCUU | 903 | 1347-1367 | AAGAGUTCCUUCCCUGGAACUUC | 1351 | 1345-1367 |
| AD-1700815.1 | GUUCCAGGGAAGGAACUCUCU | 904 | 1348-1368 | AGAGAGTUCCUUCCCUGGAACUU | 1352 | 1346-1368 |
| AD-1700816.1 | GUCCACCAUGGAGUACCUCUU | 905 | 1370-1390 | AAGAGGTACUCCAUGGUGGACCG | 1353 | 1368-1390 |
| AD-1700817.1 | CACCAUGGAGUACCUCUCAGU | 906 | 1373-1393 | ACUGAGAGGUACUCCAUGGUGGA | 1354 | 1371-1393 |
| AD-1700819.1 | CCAUGGAGUACCUCUCAGCUU | 907 | 1375-1395 | AAGCTGAGAGGUACUCCAUGGUG | 1355 | 1373-1395 |
| AD-1700821.1 | AUGGAGUACCUCUCAGCUCUU | 908 | 1377-1397 | AAGAGCTGAGAGGUACUCCAUGG | 1356 | 1375-1397 |
| AD-1700822.1 | UUACUCAGGUCAGUAUCUAAU | 909 | 1410-1430 | AUUAGATACUGACCUGAGUAAGU | 1357 | 1408-1430 |
| AD-1700824.1 | UCAGUAUCUAAUAUAAGCUCU | 910 | 1419-1439 | AGAGCUTAUAUUAGAUACUGACC | 1358 | 1417-1439 |
| AD-1700825.1 | CAGUAUCUAAUAUAAGCUCGU | 911 | 1420-1440 | ACGAGCTUAUAUUAGAUACUGAC | 1359 | 1418-1440 |
| AD-1700826.1 | AGUAUCUAAUAUAAGCUCGGU | 912 | 1421-1441 | ACCGAGCUUAUAUUAGAUACUGA | 1360 | 1419-1441 |
| AD-1700828.1 | UCUAAUAUAAGCUCGGAGUUU | 913 | 1425-1445 | AAACTCCGAGCUUAUAUUAGAUA | 1361 | 1423-1445 |
| AD-1700829.1 | CUAAUAUAAGCUCGGAGUUUU | 914 | 1426-1446 | AAAACUCCGAGCUUAUAUUAGAU | 1362 | 1424-1446 |
| AD-1700830.1 | UAAGCUCGGAGUUUGGACGGU | 915 | 1432-1452 | ACCGTCCAAACUCCGAGCUUAUA | 1363 | 1430-1452 |
| AD-1700831.1 | AAGCUCGGAGUUUGGACGGAU | 916 | 1433-1453 | AUCCGUCCAAACUCCGAGCUUAU | 1364 | 1431-1453 |
| AD-1700832.1 | GGAGUUUGGACGGAGGGUCUU | 917 | 1439-1459 | AAGACCCUCCGUCCAAACUCCGA | 1365 | 1437-1459 |
| AD-1700833.1 | UGGACGGAGGGUCUGGACCUU | 918 | 1445-1465 | AAGGTCCAGACCCUCCGUCCAAA | 1366 | 1443-1465 |
| AD-1700834.1 | AGCGACCUUUCCGUGUCUGUU | 919 | 1480-1500 | AACAGACACGGAAAGGUCGCUGG | 1367 | 1478-1500 |
| AD-1700837.1 | CAAGCGGACCAUCCGGAAAGU | 920 | 1505-1525 | ACUUTCCGGAUGGUCCGCUUGUG | 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 | CAUCCGGAAAG GCCUGACAGU | 921 | 1514-1534 | ACUGTCAGGCCUU UCCGGAUGGU | 1369 | 1512-1534 |
| AD-1700839.1 | AGGAGCUGCUA GCCAAAGCAU | 922 | 1546-1566 | AUGCTUTGGCUAG CAGCUCCUGG | 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 | AGCACUCCAUUCA 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 | AUAGCACUCCAUU 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 UCCAUUCAGC | 1388 | 1580-1602 |
| AD-1700863.1 | UGGAGUGCUAA CCCUGGUGCU | 941 | 1586-1606 | AGCACCAGGGUUA GCACUCCAUU | 1389 | 1584-1606 |
| AD-1700864.1 | GGAGUGCUAAC CCUGGUGCUU | 942 | 1587-1607 | AAGCACCAGGGUU AGCACUCCAU | 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 | CCCUGGUGCUAGAGGAGGAUU | 945 | 1597-1617 | AAUCCUCCUCUAGCACCAGGGUU | 1393 | 1595-1617 |
| AD-1700869.1 | GGUGCUAGAGGAGGAUGGAAU | 946 | 1601-1621 | AUUCCATCCUCCUCUAGCACCAG | 1394 | 1599-1621 |
| AD-1700870.1 | GUGCUAGAGGAGGAUGGAACU | 947 | 1602-1622 | AGUUCCAUCCUCCUCUAGCACCA | 1395 | 1600-1622 |
| AD-1700871.1 | CUAGAGGAGGAUGGAACUGCU | 948 | 1605-1625 | AGCAGUTCCAUCCUCCUCUAGCA | 1396 | 1603-1625 |
| AD-1700872.1 | GAGGAUGGAACUGCAGUGGAU | 949 | 1611-1631 | AUCCACTGCAGUUCCAUCCUCCU | 1397 | 1609-1631 |
| AD-1700873.1 | GGAUGGAACUGCAGUGGACAU | 950 | 1613-1633 | AUGUCCACUGCAGUUCCAUCCUC | 1398 | 1611-1633 |
| AD-1700874.1 | GAUGGAACUGCAGUGGACAGU | 951 | 1614-1634 | ACUGTCCACUGCAGUUCCAUCCU | 1399 | 1612-1634 |
| AD-1700875.1 | AUGGAACUGCAGUGGACAGUU | 952 | 1615-1635 | AACUGUCCACUGCAGUUCCAUCC | 1400 | 1613-1635 |
| AD-1700876.1 | UGGAACUGCAGUGGACAGUGU | 953 | 1616-1636 | ACACTGTCCACUGCAGUUCCAUC | 1401 | 1614-1636 |
| AD-1700877.1 | GAACUGCAGUGGACAGUGAGU | 954 | 1618-1638 | ACUCACTGUCCACUGCAGUUCCA | 1402 | 1616-1638 |
| AD-1700878.1 | ACUGCAGUGGACAGUGAGGAU | 955 | 1620-1640 | AUCCTCACUGUCCACUGCAGUUC | 1403 | 1618-1640 |
| AD-1700879.1 | CUGCAGUGGACAGUGAGGACU | 956 | 1621-1641 | AGUCCUCACUGUCCACUGCAGUU | 1404 | 1619-1641 |
| AD-1700880.1 | UGCAGUGGACAGUGAGGACUU | 957 | 1622-1642 | AAGUCCTCACUGUCCACUGCAGU | 1405 | 1620-1642 |
| AD-1700881.1 | GCAGUGGACAGUGAGGACUUU | 958 | 1623-1643 | AAAGTCCUCACUGUCCACUGCAG | 1406 | 1621-1643 |
| AD-1700882.1 | CAGUGGACAGUGAGGACUUCU | 959 | 1624-1644 | AGAAGUCCUCACUGUCCACUGCA | 1407 | 1622-1644 |
| AD-1700883.1 | AGUGGACAGUGAGGACUUCUU | 960 | 1625-1645 | AAGAAGTCCUCACUGUCCACUGC | 1408 | 1623-1645 |
| AD-1700885.1 | UGGACAGUGAGGACUUCUUCU | 961 | 1627-1647 | AGAAGAAGUCCUCACUGUCCACU | 1409 | 1625-1647 |
| AD-1700887.1 | CAGUGAGGACUUCUUCCAGCU | 962 | 1631-1651 | AGCUGGAAGAAGUCCUCACUGUC | 1410 | 1629-1651 |
| AD-1700888.1 | UGAGGACUUCUUCCAGCUGCU | 963 | 1634-1654 | AGCAGCTGGAAGAAGUCCUCACU | 1411 | 1632-1654 |
| AD-1700889.1 | GAGGACUUCUUCCAGCUGCUU | 964 | 1635-1655 | AAGCAGCUGGAAGAAGUCCUCAC | 1412 | 1633-1655 |
| AD-1700891.1 | GGACUUCUUCCAGCUGCUGGU | 965 | 1637-1657 | ACCAGCAGCUGGAAGAAGUCCUC | 1413 | 1635-1657 |
| AD-1700892.1 | UCUUCCAGCUGCUGGAGGAUU | 966 | 1642-1662 | AAUCCUCCAGCAGCUGGAAGAAG | 1414 | 1640-1662 |
| AD-1700893.1 | CAGCUGCUGGAGGAUGACACU | 967 | 1647-1667 | AGUGTCAUCCUCCAGCAGCUGGA | 1415 | 1645-1667 |
| AD-1700894.1 | GCUGCUGGAGGAUGACACGUU | 968 | 1649-1669 | AACGTGTCAUCCUCCAGCAGCUG | 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 | CUGGAGGAUGACACGUGCCUU | 969 | 1653-1673 | AAGGCACGUGUCAUCCUCCAGCA | 1417 | 1651-1673 |
| AD-1700897.1 | GGAUGACACGUGCCUGAUGGU | 970 | 1658-1678 | ACCATCAGGCACGUGUCAUCCUC | 1418 | 1656-1678 |
| AD-1700898.1 | GAUGACACGUGCCUGAUGGUU | 971 | 1659-1679 | AACCAUCAGGCACGUGUCAUCCU | 1419 | 1657-1679 |
| AD-1700899.1 | GACACGUGCCUGAUGGUGUUU | 972 | 1662-1682 | AAACACCAUCAGGCACGUGUCAU | 1420 | 1660-1682 |
| AD-1700900.1 | GCAGUCUGGUCAGAGCUGGAU | 973 | 1682-1702 | AUCCAGCUCUGACCAGACUGCAA | 1421 | 1680-1702 |
| AD-1700902.1 | UACAAGGAGUGGAGUGCUGUU | 974 | 1706-1726 | AACAGCACUCCACUCCUUGUAGG | 1422 | 1704-1726 |
| AD-1700903.1 | ACAAGGAGUGGAGUGCUGUCU | 975 | 1707-1727 | AGACAGCACUCCACUCCUUGUAG | 1423 | 1705-1727 |
| AD-1700904.1 | AAGGAGUGGAGUGCUGUCAUU | 976 | 1709-1729 | AAUGACAGCACUCCACUCCUUGU | 1424 | 1707-1729 |
| AD-1700905.1 | GGAGUGGAGUGCUGUCAUAUU | 977 | 1711-1731 | AAUATGACAGCACUCCACUCCUU | 1425 | 1709-1731 |
| AD-1700906.1 | UGGAGUGCUGUCAUAUGGCCU | 978 | 1715-1735 | AGGCCATAUGACAGCACUCCACU | 1426 | 1713-1735 |
| AD-1700907.1 | GGAGUGCUGUCAUAUGGCCUU | 979 | 1716-1736 | AAGGCCAUAUGACAGCACUCCAC | 1427 | 1714-1736 |
| AD-1700908.1 | GAGUGCUGUCAUAUGGCCUGU | 980 | 1717-1737 | ACAGGCCAUAUGACAGCACUCCA | 1428 | 1715-1737 |
| AD-1700909.1 | AGUGCUGUCAUAUGGCCUGGU | 981 | 1718-1738 | ACCAGGCCAUAUGACAGCACUCC | 1429 | 1716-1738 |
| AD-1700912.1 | GCUGUCAUAUGGCCUGGGACU | 982 | 1721-1741 | AGUCCCAGGCCAUAUGACAGCAC | 1430 | 1719-1741 |
| AD-1700913.1 | CUGUCAUAUGGCCUGGGACGU | 983 | 1722-1742 | ACGUCCCAGGCCAUAUGACAGCA | 1431 | 1720-1742 |
| AD-1700915.1 | CAAGCACAGCAAGGACAUCGU | 984 | 1751-1771 | ACGATGTCCUUGCUGUGCUUGGG | 1432 | 1749-1771 |
| AD-1700916.1 | AUUCACCUUUGACGUGUACAU | 985 | 1775-1795 | AUGUACACGUCAAAGGUGAAUCG | 1433 | 1773-1795 |
| AD-1700917.1 | UUCACCUUUGACGUGUACAAU | 986 | 1776-1796 | AUUGTACACGUCAAAGGUGAAUC | 1434 | 1774-1796 |
| AD-1700918.1 | UCACCUUUGACGUGUACAAGU | 987 | 1777-1797 | ACUUGUACACGUCAAAGGUGAAU | 1435 | 1775-1797 |
| AD-1700919.1 | CACCUUUGACGUGUACAAGCU | 988 | 1778-1798 | AGCUTGTACACGUCAAAGGUGAA | 1436 | 1776-1798 |
| AD-1700920.1 | CCUUUGACGUGUACAAGCAAU | 989 | 1780-1800 | AUUGCUTGUACACGUCAAAGGUG | 1437 | 1778-1800 |
| AD-1700922.1 | UGACGUGUACAAGCAAAACCU | 990 | 1784-1804 | AGGUTUTGCUUGUACACGUCAAA | 1438 | 1782-1804 |
| AD-1700923.1 | GACGUGUACAAGCAAAACCCU | 991 | 1785-1805 | AGGGTUTUGCUUGUACACGUCAA | 1439 | 1783-1805 |
| AD-1700924.1 | ACGUGUACAAGCAAAACCCUU | 992 | 1786-1806 | AAGGGUTUUGCUUGUACACGUCA | 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 UCGAGACCUU | 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 AUGUCAAAGU | 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 UCAAAGCCAU | 1004 | 1820-1840 | AUGGCUTUGACAU UCAGGCUGCC | 1452 | 1818-1840 |
| AD-1700944.1 | AGCCUGAAUGU CAAAGCCACU | 1005 | 1821-1841 | AGUGGCTUUGACA UUCAGGCUGC | 1453 | 1819-1841 |
| AD-1700947.1 | AUGUCAAAGCC 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 | AACAACTCAUAGA 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 | CUAUGAGUUGUGACUUUCAAU | 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 | AGAGTACUUUCUUUGGGCCAAGU | 1471 | 1880-1902 |
| AD-1700970.1 | UGGCCCAAAGAAAGUACUCAU | 1024 | 1883-1903 | AUGAGUACUUUCUUUGGGCCAAG | 1472 | 1881-1903 |
| AD-1700971.1 | GGCCCAAAGAAAGUACUCAGU | 1025 | 1884-1904 | ACUGAGTACUUUCUUUGGGCCAA | 1473 | 1882-1904 |
| AD-1700973.1 | CCCAAAGAAAGUACUCAGGGU | 1026 | 1886-1906 | ACCCTGAGUACUUUCUUUGGGCC | 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 | UUCGUUGGACCUCCACACUGU | 1033 | 1912-1932 | ACAGTGTGGAGGUCCAACGAAGG | 1481 | 1910-1932 |
| AD-1700984.1 | CGUUGGACCUCCACACUGCUU | 1034 | 1914-1934 | AAGCAGTGUGGAGGUCCAACGAA | 1482 | 1912-1934 |
| AD-1700985.1 | GACCUCCACACUGCUGCAAGU | 1035 | 1919-1939 | ACUUGCAGCAGUGUGGAGGUCCA | 1483 | 1917-1939 |
| AD-1700986.1 | GGCCUGGGCCAUAUGUUGCUU | 1036 | 1938-1958 | AAGCAACAUAUGGCCCAGGCCUU | 1484 | 1936-1958 |
| AD-1700988.1 | GGGCCAUAUGUUGCUGGGAAU | 1037 | 1943-1963 | AUUCCCAGCAACAUAUGGCCCAG | 1485 | 1941-1963 |
| AD-1700989.1 | GGCCAUAUGUUGCUGGGAAUU | 1038 | 1944-1964 | AAUUCCCAGCAACAUAUGGCCCA | 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 | UGUUGCUGGGAAUUUCCUCCU | 1041 | 1951-1971 | AGGAGGAAAUUCCCAGCAACAUA | 1489 | 1949-1971 |
| AD-1700995.1 | UGGGAAUUUCCUCCACCCUUU | 1042 | 1957-1977 | AAAGGGTGGAGGAAAUUCCCAGC | 1490 | 1955-1977 |
| AD-1700997.1 | UUCCUCCACCCUUCGUCAUGU | 1043 | 1964-1984 | ACAUGACGAAGGGUGGAGGAAAU | 1491 | 1962-1984 |
| AD-1700998.1 | AAGGGCCGCCUCCAUUCCUAU | 1044 | 2013-2033 | AUAGGAAUGGAGGCGGCCCUUCU | 1492 | 2011-2033 |
| AD-1701001.1 | GCCGCCUCCAUUCCUACUAAU | 1045 | 2017-2037 | AUUAGUAGGAAUGGAGGCGGCCC | 1493 | 2015-2037 |
| AD-1701002.1 | CCGCCUCCAUUCCUACUAAGU | 1046 | 2018-2038 | ACUUAGTAGGAAUGGAGGCGGCC | 1494 | 2016-2038 |
| AD-1701003.1 | CCCACUGCAAAGACUAUGACU | 1047 | 2076-2096 | AGUCAUAGUCUUUGCAGUGGGUC | 1495 | 2074-2096 |
| AD-1701004.1 | CCACUGCAAAGACUAUGACAU | 1048 | 2077-2097 | AUGUCATAGUCUUUGCAGUGGGU | 1496 | 2075-2097 |
| AD-1701005.1 | CACUGCAAAGACUAUGACAGU | 1049 | 2078-2098 | ACUGTCAUAGUCUUUGCAGUGGG | 1497 | 2076-2098 |
| AD-1701007.1 | AAGACUAUGACAGCAUCAAAU | 1050 | 2085-2105 | AUUUGATGCUGUCAUAGUCUUUG | 1498 | 2083-2105 |
| AD-1701009.1 | GACAGCAUCAAAUUUCAGGAU | 1051 | 2093-2113 | AUCCTGAAAUUUGAUGCUGUCAU | 1499 | 2091-2113 |
| AD-1701011.1 | CAGCAUCAAAUUUCAGGACCU | 1052 | 2095-2115 | AGGUCCTGAAAUUUGAUGCUGUC | 1500 | 2093-2115 |
| AD-1701012.1 | AGCAUCAAAUUUCAGGACCUU | 1053 | 2096-2116 | AAGGTCCUGAAAUUUGAUGCUGU | 1501 | 2094-2116 |
| AD-1701013.1 | GCAUCAAAUUUCAGGACCUGU | 1054 | 2097-2117 | ACAGGUCCUGAAAUUUGAUGCUG | 1502 | 2095-2117 |
| AD-1701014.1 | CAUCAAAUUUCAGGACCUGCU | 1055 | 2098-2118 | AGCAGGTCCUGAAAUUUGAUGCU | 1503 | 2096-2118 |
| AD-1701016.1 | AUUUCAGGACCUGCAGACAGU | 1056 | 2104-2124 | ACUGTCTGCAGGUCCUGAAAUUU | 1504 | 2102-2124 |
| AD-1701017.1 | UUUCAGGACCUGCAGACAGUU | 1057 | 2105-2125 | AACUGUCUGCAGGUCCUGAAAUU | 1505 | 2103-2125 |
| AD-1701019.1 | CAGGACCUGCAGACAGUACAU | 1058 | 2108-2128 | AUGUACTGUCUGCAGGUCCUGAA | 1506 | 2106-2128 |
| AD-1701020.1 | GACCUGCAGACAGUACAGGCU | 1059 | 2111-2131 | AGCCTGTACUGUCUGCAGGUCCU | 1507 | 2109-2131 |
| AD-1701022.1 | CCUGCAGACAGUACAGGCUAU | 1060 | 2113-2133 | AUAGCCTGUACUGUCUGCAGGUC | 1508 | 2111-2133 |
| AD-1701023.1 | CUGCAGACAGUACAGGCUAGU | 1061 | 2114-2134 | ACUAGCCUGUACUGUCUGCAGGU | 1509 | 2112-2134 |
| AD-1701024.1 | UGCAGACAGUACAGGCUAGAU | 1062 | 2115-2135 | AUCUAGCCUGUACUGUCUGCAGG | 1510 | 2113-2135 |
| AD-1701026.1 | CAGACAGUACAGGCUAGAUAU | 1063 | 2117-2137 | AUAUCUAGCCUGUACUGUCUGCA | 1511 | 2115-2137 |
| AD-1701027.1 | GACAGUACAGGCUAGAUAACU | 1064 | 2119-2139 | AGUUAUCUAGCCUGUACUGUCUG | 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 | CCUAGCCACCUUUCUGGCCUU | 1065 | 2243-2263 | AAGGCCAGAAAGGUGGCUAGGAA | 1513 | 2241-2263 |
| AD-1701031.1 | CUUUCUGGCCUCCCACUUGCU | 1066 | 2252-2272 | AGCAAGTGGGAGGCCAGAAAGGU | 1514 | 2250-2272 |
| AD-1701032.1 | UCUGGCCUCCCACUUGCCCUU | 1067 | 2255-2275 | AAGGGCAAGUGGGAGGCCAGAAA | 1515 | 2253-2275 |
| AD-1701033.1 | GGCAUCUUGAUCCCAGCCUGU | 1068 | 2302-2322 | ACAGGCTGGGAUCAAGAUGCCUG | 1516 | 2300-2322 |
| AD-1701034.1 | GCAUCUUGAUCCCAGCCUGAU | 1069 | 2303-2323 | AUCAGGCUGGGAUCAAGAUGCCU | 1517 | 2301-2323 |
| AD-1701035.1 | ACCAAUGCCUCCUGUCCCUAU | 1070 | 2345-2365 | AUAGGGACAGGAGGCAUUGGUAG | 1518 | 2343-2365 |
| AD-1701037.1 | AUGCCUCCUGUCCCUAAACUU | 1071 | 2349-2369 | AAGUTUAGGGACAGGAGGCAUUG | 1519 | 2347-2369 |
| AD-1701038.1 | GCCUCCUGUCCCUAAACUCCU | 1072 | 2351-2371 | AGGAGUTUAGGGACAGGAGGCAU | 1520 | 2349-2371 |
| AD-1720280.1 | AAAACCCUCGAGACCUCUUUU | 1073 | 1798-1818 | AAAAGAGGUCUCGAGGGUUUUGC | 1521 | 1796-1818 |
| AD-1720281.1 | CUGGGCUGAGAUCCCAGGUUU | 1074 | 1272-1292 | AAACCTGGGAUCUCAGCCCAGGA | 1522 | 1270-1292 |
| AD-1720282.1 | GUGGACAGUGAGGACUUCUUU | 1075 | 1626-1646 | AAAGAAGUCCUCACUGUCCACUG | 1523 | 1624-1646 |
| AD-1720283.1 | CAAAACCCUCGAGACCUCUUU | 1076 | 1797-1817 | AAAGAGGTCUCGAGGGUUUUGCU | 1524 | 1795-1817 |
| AD-1720284.1 | CUGAAUGUCAAAGCCACAUUU | 1077 | 1824-1844 | AAATGTGGCUUTGACAUUCAGGC | 1525 | 1822-1844 |
| AD-1720285.1 | GGCUCUACUCUAUGAGUUGUU | 1078 | 1849-1869 | AACAACTCAUAGAGUAGAGCCCG | 1526 | 1847-1869 |
| AD-1720286.1 | AAUGGAGUGCUAACCCUGGUU | 1079 | 1584-1604 | AACCAGGGUUAGCACUCCAUUCA | 1527 | 1582-1604 |
| AD-1720287.1 | AAUGGAGUGCUAACCUUGGUU | 1080 | 1584-1604 | AACCAAGGUUAGCACUCCAUUCA | 1528 | 1582-1604 |
| AD-1720288.1 | AAUGGAGUGCUAACUCUGGUU | 1081 | 1584-1604 | AACCAGAGUUAGCACUCCAUUCA | 1529 | 1582-1604 |
| AD-1720289.1 | ACCCUACUGCUGAAUGGAGUU | 1082 | 1572-1592 | AACUCCAUUCAGCAGUAGGGUCU | 1530 | 1570-1592 |
| AD-1720290.1 | ACCUGCAGACAGUACAGGCUU | 1083 | 2112-2132 | AAGCCTGUACUGUCUGCAGGUCC | 1531 | 2110-2132 |
| AD-1720291.1 | CUGGGAAUUUCCUCCACCCUU | 1084 | 1956-1976 | AAGGGTGGAGGAAAUUCCCAGCA | 1532 | 1954-1976 |
| AD-1720292.1 | GGGCCGCCUCCAUUCCUACUU | 1085 | 2015-2035 | AAGUAGGAAUGGAGGCGGCCCUU | 1533 | 2013-2035 |
| AD-1720293.1 | UUCUACGGGCUCUACUCUAUU | 1086 | 1842-1862 | AAUAGAGUAGAGCCCGUAGAAUG | 1534 | 1840-1862 |
| AD-1720294.1 | GCAGACAGUACAGGCUAGAUU | 1087 | 2116-2136 | AAUCUAGCCUGTACUGUCUGCAG | 1535 | 2114-2136 |
| AD-1720295.1 | UGGAGACCCUACUGCUGAAUU | 1088 | 1567-1587 | AAUTCAGCAGUAGGGUCUCCAAU | 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 | ACCCAGGCCAUAU 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 | GGAAUUUCCUC CACCCUUCGU | 1099 | 1959-1979 | ACGAAGGGUGGA GGAAAUUCCCA | 1547 | 1957-1979 |
| AD-1720307.1 | GGAAUUUCCUC CACCUUUCGU | 1100 | 1959-1979 | ACGAAAGGUGGA GGAAAUUCCCA | 1548 | 1957-1979 |
| AD-1720308.1 | GGAAUUUCCUC 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 | UCAAAUUUCAG 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 UGUCAAAGCU | 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 | UGCUGAAUGGAGUGCUAACCU | 1113 | 1579-1599 | AGGTUAGCACUCCAUUCAGCAGU | 1561 | 1577-1599 |
| AD-1720371.1 | UCAGGACCUGCAGACAGUACU | 1114 | 2107-2127 | AGUACTGUCUGCAGGUCCUGAAA | 1562 | 2105-2127 |
| AD-1720372.1 | ACAGCAUCAAAUUUCAGGACU | 1115 | 2094-2114 | AGUCCTGAAAUTUGAUGCUGUCA | 1563 | 2092-2114 |
| AD-1720373.1 | UUGCUGGGAAUUUCCUCCACU | 1116 | 1953-1973 | AGUGGAGGAAATUCCCAGCAACA | 1564 | 1951-1973 |
| AD-1720374.1 | AAUGCCUCCUGUCCCUAAACU | 1117 | 2348-2368 | AGUTUAGGGACAGGAGGCAUUGG | 1565 | 2346-2368 |
| AD-1720375.1 | UUGACGUGUACAAGCAAAACU | 1118 | 1783-1803 | AGUTUTGCUUGTACACGUCAAAG | 1566 | 1781-1803 |
| AD-1720376.1 | GGCCGCCUCCAUUCCUACUAU | 1119 | 2016-2036 | ATAGUAGGAAUGGAGGCGGCCCU | 1567 | 2014-2036 |
| AD-1720377.1 | ACUCAGGUCAGUAUCUAAUAU | 1120 | 1412-1432 | ATATUAGAUACTGACCUGAGUAA | 1568 | 1410-1432 |
| AD-1720378.1 | UGGGCCAUAUGUUGCUGGGAU | 1121 | 1942-1962 | ATCCCAGCAACAUAUGGCCCAGG | 1569 | 1940-1962 |
| AD-1720379.1 | UGCUGUCAUAUGGCCUGGGAU | 1122 | 1720-1740 | ATCCCAGGCCATAUGACAGCACU | 1570 | 1718-1740 |
| AD-1720380.1 | UGAGUUGUGACUUUCAAGGAU | 1123 | 1861-1881 | ATCCUTGAAAGTCACAACUCAUA | 1571 | 1859-1881 |
| AD-1720381.1 | AGGCCCAAGCACAGCAAGGAU | 1124 | 1746-1766 | ATCCUTGCUGUGCUUGGGCCUCU | 1572 | 1744-1766 |
| AD-1720382.1 | UGUACAAGCAAAACCCUCGAU | 1125 | 1789-1809 | ATCGAGGGUUUTGCUUGUACACG | 1573 | 1787-1809 |
| AD-1720383.1 | UGUACAAGCAAAACCUUCGAU | 1126 | 1789-1809 | ATCGAAGGUUUTGCUUGUACACG | 1574 | 1787-1809 |
| AD-1720384.1 | UGUACAAGCAAAACUCUCGAU | 1127 | 1789-1809 | ATCGAGAGUUUTGCUUGUACACG | 1575 | 1787-1809 |
| AD-1720385.1 | UCUAUGAGUUGUGACUUUCAU | 1128 | 1857-1877 | ATGAAAGUCACAACUCAUAGAGU | 1576 | 1855-1877 |
| AD-1720386.1 | AAAGACUAUGACAGCAUCAAU | 1129 | 2084-2104 | ATUGATGCUGUCAUAGUCUUUGC | 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 | gsusccugggCfUfGfagaucccaguL96 | 1578 | asdCsugdGgdAucucdAgCfccaggacsasa | 2026 | GGAAGGAAAGAGCAGAUCCCAGG | 2474 |
| AD-1699867.1 | csusgggcugAfGfAfucccagguuuL96 | 1579 | asdAsacdCudGggaudCuCfagcccagsgsa | 2027 | AGGAAAGAGCAGAUCCCAGGUUU | 2475 |
| AD-1699868.1 | usgsggcugaGfAfUfcccagguuuuL96 | 1580 | asdAsaadCcdTgggadTcUfcagcccasgsg | 2028 | GGAAAGAGCAGAUCCCAGGUUUG | 2476 |
| AD-1699869.1 | gsgsgcugagAfUfCfccagguuuguL96 | 1581 | asdCsaadAcdCugggdAuCfucagcccsasg | 2029 | GAAAGAGCAGAUCCCAGGUUUGU | 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 | gsgscugagaUfCfCfcagguuuguuL96 | 1582 | asdAscadAadCcuggdGaUfcucagccscsa | 2030 | AAAGAGCAGAUCCCAGGUUUGUA | 2478 |
| AD-1699871.1 | gscsugagauCfCfCfagguuuguauL96 | 1583 | asdTsacdAadAccugdGgAfucucagcscsc | 2031 | AAGAGCAGAUCCCAGGUUUGUAA | 2479 |
| AD-1699872.1 | csusgagaucCfCfAfgguuuguaauL96 | 1584 | asdTsuadCadAaccudGgGfaucucagscsc | 2032 | AGAGCAGAUCCCAGGUUUGUAAC | 2480 |
| AD-1699873.1 | usgsagauccCfAfGfguuuguaacuL96 | 1585 | asdGsuudAcdAaaccdTgGfgaucucasgsc | 2033 | GAGCAGAUCCCAGGUUUGUAACA | 2481 |
| AD-1699875.1 | asgsaucccaGfGfUfuuguaacaguL96 | 1586 | asdCsugdTudAcaaadCcUfgggaucuscsa | 2034 | GCAGAUCCCAGGUUUGUAACAGA | 2482 |
| AD-1699882.1 | asgsguuuguAfAfCfagaaaacacuL96 | 1587 | asdGsugdTudTucugdTuAfcaaaccusgsg | 2035 | CCAGGUUUGUAACAGAAAACACC | 2483 |
| AD-1699891.1 | ascsagaaaaCfAfCfcacuaaagcuL96 | 1588 | asdGscudTudAguggdTgUfuuucugususa | 2036 | UAACAGAAAACACCACUAAAGCC | 2484 |
| AD-1699892.1 | csasgaaaacAfCfCfacuaaagccuL96 | 1589 | asdGsgcdTudTagugdGuGfuuuucugsusu | 2037 | AACAGAAAACACCACUAAAGCCC | 2485 |
| AD-1699904.1 | asgsaaccacCfCfAfgcccagaaguL96 | 1590 | asdCsuudCudGggcudGgGfugguucuscsu | 2038 | AGAGAACCACCCAGCCCAGAAGU | 2486 |
| AD-1699907.1 | ascscacccaGfCfCfcagaaguucuL96 | 1591 | asdGsaadCudTcuggdGcUfggguggususc | 2039 | GAACCACCCAGCCCAGAAGUUCC | 2487 |
| AD-1699948.1 | csasccauggAfGfUfaccucucaguL96 | 1592 | asdCsugdAgdAgguadCuCfcauggugsgsa | 2040 | UCCACCAUGGAGUACCUCUCAGC | 2488 |
| AD-1699949.1 | ascscauggaGfUfAfccucucagcuL96 | 1593 | asdGscudGadGaggudAcUfccauggusgsg | 2041 | CCACCAUGGAGUACCUCUCAGCU | 2489 |
| AD-1699951.1 | csasuggaguAfCfCfucucagcucuL96 | 1594 | asdGsagdCudGagagdGuAfcuccaugsgsu | 2042 | ACCAUGGAGUACCUCUCAGCUCU | 2490 |
| AD-1699964.1 | csusuacucaGfGfUfcaguaucuauL96 | 1595 | asdTsagdAudAcugadCcUfgaguaagsusc | 2043 | GACUUACUCAGGUCAGUAUCUAA | 2491 |
| AD-1699967.1 | ascsucagguCfAfGfuaucuaauauL96 | 1596 | asdTsaudTadGauacdTgAfccugagusasa | 2044 | UUACUCAGGUCAGUAUCUAAUAU | 2492 |
| AD-1699970.1 | csasggucagUfAfUfcuaauauaauL96 | 1597 | asdTsuadTadTuagadTaCfugaccugsasg | 2045 | CUCAGGUCAGUAUCUAAUAUAAG | 2493 |
| AD-1699971.1 | asgsgucaguAfUfCfuaauauaaguL96 | 1598 | asdCsuudAudAuuagdAuAfcugaccusgsa | 2046 | UCAGGUCAGUAUCUAAUAUAAGC | 2494 |
| AD-1699972.1 | gsgsucaguaUfCfUfaauauaagcuL96 | 1599 | asdGscudTadTauuadGaUfacugaccsusg | 2047 | CAGGUCAGUAUCUAAUAUAAGCU | 2495 |
| AD-1699973.1 | gsuscaguauCfUfAfauauaagcuuL96 | 1600 | asdAsgcdTudAuauudAgAfuacugacscsu | 2048 | AGGUCAGUAUCUAAUAUAAGCUC | 2496 |
| AD-1699976.1 | asgsuaucuaAfUfAfuaagcucgguL96 | 1601 | asdCscgdAgdCuuaudAuUfagauacusgsa | 2049 | UCAGUAUCUAAUAUAAGCUCGGA | 2497 |
| AD-1699977.1 | gsusaucuaaUfAfUfaagcucggauL96 | 1602 | asdTsccdGadGcuuadTaUfuagauacsusg | 2050 | CAGUAUCUAAUAUAAGCUCGGAG | 2498 |
| AD-1699978.1 | usasucuaauAfUfAfagcucggaguL96 | 1603 | asdCsucdCgdAgcuudAuAfuuagauascsu | 2051 | AGUAUCUAAUAUAAGCUCGGAGU | 2499 |
| AD-1699979.1 | asuscuaauaUfAfAfgcucggaguuL96 | 1604 | asdAscudCcdGagcudTaUfauuagausasc | 2052 | GUAUCUAAUAUAAGCUCGGAGUU | 2500 |
| AD-1699982.1 | usasauauaaGfCfUfcggaguuuguL96 | 1605 | asdCsaadAcdTccgadGcUfuauauuasgsa | 2053 | UCUAAUAUAAGCUCGGAGUUUGG | 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 | asasuauaagCfUfCfggaguuugguL96 | 1606 | asdCscadAadCuccgdAgCfuuauauusasg | 2054 | CUAAUAUAAGCUCGGAGUUUGGA | 2502 |
| AD-1699984.1 | asusauaagcUfCfGfgaguuuggauL96 | 1607 | asdTsccdAadAcuccdGaGfcuuauaususa | 2055 | UAAUAUAAGCUCGGAGUUUGGAC | 2503 |
| AD-1699985.1 | usasuaagcuCfGfGfaguuuggacuL96 | 1608 | asdGsucdCadAacucdCgAfgcuuauasusu | 2056 | AAUAUAAGCUCGGAGUUUGGACG | 2504 |
| AD-1699986.1 | asusaagcucGfGfAfguuuggacguL96 | 1609 | asdCsgudCcdAaacudCcGfagcuuausasu | 2057 | AUAUAAGCUCGGAGUUUGGACGG | 2505 |
| AD-1699987.1 | usasagcucgGfAfGfuuuggacgguL96 | 1610 | asdCscgdTcdCaaacdTcCfgagcuuasusa | 2058 | UAUAAGCUCGGAGUUUGGACGGA | 2506 |
| AD-1699988.1 | asasgcucggAfGfUfuuggacggauL96 | 1611 | asdTsccdGudCcaaadCuCfcgagcuusasu | 2059 | AUAAGCUCGGAGUUUGGACGGAG | 2507 |
| AD-1699989.1 | asgscucggaGfUfUfuggacggaguL96 | 1612 | asdCsucdCgdTccaadAcUfccgagcususa | 2060 | UAAGCUCGGAGUUUGGACGGAGG | 2508 |
| AD-1699990.1 | gscsucggagUfUfUfggacggagguL96 | 1613 | asdCscudCcdGuccadAaCfuccgagcsusu | 2061 | AAGCUCGGAGUUUGGACGGAGGG | 2509 |
| AD-1700015.1 | asgscgaccuUfUfCfcgugucuguuL96 | 1614 | asdAscadGadCacggdAaAfggucgcusgsg | 2062 | CCAGCGACCUUUCCGUGUCUGUG | 2510 |
| AD-1700035.1 | gsasucacaaGfCfGfgaccauccguL96 | 1615 | asdCsggdAudGguccdGcUfugugaucsasc | 2063 | GUGAUCACAAGCGGACCAUCCGG | 2511 |
| AD-1700037.1 | uscsacaagcGfGfAfccauccggauL96 | 1616 | asdTsccdGgdAuggudCcGfcuugugasusc | 2064 | GAUCACAAGCGGACCAUCCGGAA | 2512 |
| AD-1700038.1 | csascaagcgGfAfCfcauccggaauL96 | 1617 | asdTsucdCgdGauggdTcCfgcuugugsasu | 2065 | AUCACAAGCGGACCAUCCGGAAA | 2513 |
| AD-1700039.1 | ascsaagcggAfCfCfauccggaaauL96 | 1618 | asdTsuudCcdGgaugdGuCfcgcuugusgsa | 2066 | UCACAAGCGGACCAUCCGGAAAG | 2514 |
| AD-1700040.1 | csasagcggaCfCfAfuccggaaaguL96 | 1619 | asdCsuudTcdCggaudGgUfccgcuugsusg | 2067 | CACAAGCGGACCAUCCGGAAAGG | 2515 |
| AD-1700041.1 | asasgcggacCfAfUfccggaaagguL96 | 1620 | asdCscudTudCcggadTgGfuccgcuusgsu | 2068 | ACAAGCGGACCAUCCGGAAAGGC | 2516 |
| AD-1700077.1 | gsgsagcugcUfAfGfccaaagcauuL96 | 1621 | asdAsugdCudTuggcdTaGfcagcuccsusg | 2069 | CAGGAGCUGCUAGCCAAAGCAUU | 2517 |
| AD-1700080.1 | gscsugcuagCfCfAfaagcauugguL96 | 1622 | asdCscadAudGcuuudGgCfuagcagcsusc | 2070 | GAGCUGCUAGCCAAAGCAUUGGA | 2518 |
| AD-1700081.1 | csusgcuagcCfAfAfagcauuggauL96 | 1623 | asdTsccdAadTgcuudTgGfcuagcagscsu | 2071 | AGCUGCUAGCCAAAGCAUUGGAG | 2519 |
| AD-1700082.1 | usgscuagccAfAfAfgcauuggaguL96 | 1624 | asdCsucdCadAugcudTuGfgcuagcasgsc | 2072 | GCUGCUAGCCAAAGCAUUGGAGA | 2520 |
| AD-1700083.1 | gscsuagccaAfAfGfcauuggagauL96 | 1625 | asdTscudCcdAaugcdTuUfggcuagcsasg | 2073 | CUGCUAGCCAAAGCAUUGGAGAC | 2521 |
| AD-1700091.1 | asasgcauugGfAfGfacccuacuguL96 | 1626 | asdCsagdTadGggucdTcCfaaugcuususg | 2074 | CAAAGCAUUGGAGACCCUACUGC | 2522 |
| AD-1700092.1 | asgscauuggAfGfAfcccuacugcuL96 | 1627 | asdGscadGudAgggudCuCfcaaugcususu | 2075 | AAAGCAUUGGAGACCCUACUGCU | 2523 |
| AD-1700094.1 | csasuuggagAfCfCfcuacugcuguL96 | 1628 | asdCsagdCadGuaggdGuCfuccaaugscsu | 2076 | AGCAUUGGAGACCCUACUGCUGA | 2524 |
| AD-1700097.1 | usgsgagaccCfUfAfcugcugaauuL96 | 1629 | asdAsuudCadGcagudAgGfgucuccasasu | 2077 | AUUGGAGACCCUACUGCUGAAUG | 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 | gsasgacccuAfCfUfgcugaaugguL96 | 1630 | asdCscadTudCagcadGuAfgggucucscsa | 2078 | UGGAGACCCUACUGCUGAAUGGA | 2526 |
| AD-1700100.1 | asgsacccuaCfUfGfcugaauggauL96 | 1631 | asdTsccdAudTcagcdAgUfagggucuscsc | 2079 | GGAGACCCUACUGCUGAAUGGAG | 2527 |
| AD-1700101.1 | gsascccuacUfGfCfugaauggaguL96 | 1632 | asdCsucdCadTucagdCaGfuagggucsusc | 2080 | GAGACCCUACUGCUGAAUGGAGU | 2528 |
| AD-1700104.1 | cscsuacugcUfGfAfauggagugcuL96 | 1633 | asdGscadCudCcauudCaGfcaguaggsgsu | 2081 | ACCCUACUGCUGAAUGGAGUGCU | 2529 |
| AD-1700109.1 | usgscugaauGfGfAfgugcuaaccuL96 | 1634 | asdGsgudTadGcacudCcAfuucagcasgsu | 2082 | ACUGCUGAAUGGAGUGCUAACCC | 2530 |
| AD-1700112.1 | usgsaauggaGfUfGfcuaacccuguL96 | 1635 | asdCsagdGgdTuagcdAcUfccauucasgsc | 2083 | GCUGAAUGGAGUGCUAACCCUGG | 2531 |
| AD-1700113.1 | gsasauggagUfGfCfuaacccugguL96 | 1636 | asdCscadGgdGuuagdCaCfuccauucsasg | 2084 | CUGAAUGGAGUGCUAACCCUGGU | 2532 |
| AD-1700114.1 | asasuggaguGfCfUfaacccugguuL96 | 1637 | asdAsccdAgdGguuadGcAfcuccauuscsa | 2085 | UGAAUGGAGUGCUAACCCUGGUG | 2533 |
| AD-1700115.1 | asusggagugCfUfAfacccugguguL96 | 1638 | asdCsacdCadGgguudAgCfacuccaususc | 2086 | GAAUGGAGUGCUAACCCUGGUGC | 2534 |
| AD-1700116.1 | usgsgagugcUfAfAfcccuggugcuL96 | 1639 | asdGscadCcdAgggudTaGfcacuccasusu | 2087 | AAUGGAGUGCUAACCCUGGUGCU | 2535 |
| AD-1700121.1 | usgscuaaccCfUfGfgugcuagaguL96 | 1640 | asdCsucdTadGcaccdAgGfguuagcascsu | 2088 | AGUGCUAACCCUGGUGCUAGAGG | 2536 |
| AD-1700122.1 | gscsuaacccUfGfGfugcuagagguL96 | 1641 | asdCscudCudAgcacdCaGfgguuagcsasc | 2089 | GUGCUAACCCUGGUGCUAGAGGA | 2537 |
| AD-1700125.1 | asascccuggUfGfCfuagaggagguL96 | 1642 | asdCscudCcdTcuagdCaCfcaggguusasg | 2090 | CUAACCCUGGUGCUAGAGGAGGA | 2538 |
| AD-1700148.1 | gsasacugcaGfUfGfgacagugaguL96 | 1643 | asdCsucdAcdTguccdAcUfgcaguucscsa | 2091 | UGGAACUGCAGUGGACAGUGAGG | 2539 |
| AD-1700149.1 | asascugcagUfGfGfacagugagguL96 | 1644 | asdCscudCadCugucdCaCfugcaguuscsc | 2092 | GGAACUGCAGUGGACAGUGAGGA | 2540 |
| AD-1700156.1 | gsusggacagUfGfAfggacuucuuuL96 | 1645 | asdAsagdAadGuccudCaCfuguccacsusg | 2093 | CAGUGGACAGUGAGGACUUCUUC | 2541 |
| AD-1700158.1 | gsgsacagugAfGfGfacuucuuccuL96 | 1646 | asdGsgadAgdAagucdCuCfacuguccsasc | 2094 | GUGGACAGUGAGGACUUCUUCCA | 2542 |
| AD-1700159.1 | gsascagugaGfGfAfcuucuuccauL96 | 1647 | asdTsggdAadGaagudCcUfcacugucscsa | 2095 | UGGACAGUGAGGACUUCUUCCAG | 2543 |
| AD-1700160.1 | ascsagugagGfAfCfuucuuccaguL96 | 1648 | asdCsugdGadAgaagdTcCfucacugucscsc | 2096 | GGACAGUGAGGACUUCUUCCAGC | 2544 |
| AD-1700166.1 | asgsgacuucUfUfCfcagcugcuguL96 | 1649 | asdCsagdCadGcuggdAaGfaaguccuscsa | 2097 | UGAGGACUUCUUCCAGCUGCUGG | 2545 |
| AD-1700167.1 | gsgsacuucuUfCfCfagcugcugguL96 | 1650 | asdCscadGcdAgcugdGaAfgaaguccsusc | 2098 | GAGGACUUCUUCCAGCUGCUGGA | 2546 |
| AD-1700180.1 | csusgcuggaGfGfAfugacacguguL96 | 1651 | asdCsacdGudGucaudCcUfccagcagscsu | 2099 | AGCUGCUGGAGGAUGACACGUGC | 2547 |
| AD-1700187.1 | asgsgaugacAfCfGfugccugauguL96 | 1652 | asdCsaudCadGgcacdGuGfucauccuscsc | 2100 | GGAGGAUGACACGUGCCUGAUGG | 2548 |
| AD-1700188.1 | gsgsaugacaCfGfUfgccugaugguL96 | 1653 | asdCscadTcdAggcadCgUfgucauccsusc | 2101 | GAGGAUGACACGUGCCUGAUGGU | 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 | gsasugacacGfUfGfccugaugguuL96 | 1654 | asdAsccdAudCaggcdAcGfugucaucscsu | 2102 | AGGAUGACACGUGCCUGAUGGUG | 2550 |
| AD-1700190.1 | asusgacacgUfGfCfcugaugguguL96 | 1655 | asdCsacdCadTcaggdCaCfgugucauscsc | 2103 | GGAUGACACGUGCCUGAUGGUGU | 2551 |
| AD-1700191.1 | usgsacacguGfCfCfugaugguguuL96 | 1656 | asdAscadCcdAucagdGcAfcgugucasusc | 2104 | GAUGACACGUGCCUGAUGGUGUU | 2552 |
| AD-1700193.1 | ascsacgugcCfUfGfaugguguuguL96 | 1657 | asdCsaadCadCcaucdAgGfcacguguscsa | 2105 | UGACACGUGCCUGAUGGUGUUGC | 2553 |
| AD-1700213.1 | csasgucuggUfCfAfgagcuggaguL96 | 1658 | asdCsucdCadGcucudGaCfcagacugscsa | 2106 | UGCAGUCUGGUCAGAGCUGGAGC | 2554 |
| AD-1700242.1 | gsasguggagUfGfCfugucauauguL96 | 1659 | asdCsaudAudGacagdCaCfuccacucscsu | 2107 | AGGAGUGGAGUGCUGUCAUAUGG | 2555 |
| AD-1700243.1 | asgsuggaguGfCfUfgucauaugguL96 | 1660 | asdCscadTadTgacadGcAfcuccacuscsc | 2108 | GGAGUGGAGUGCUGUCAUAUGGC | 2556 |
| AD-1700244.1 | gsusggagugCfUfGfucauauggcuL96 | 1661 | asdGsccdAudAugacdAgCfacuccacsusc | 2109 | GAGUGGAGUGCUGUCAUAUGGCC | 2557 |
| AD-1700245.1 | usgsgagugcUfGfUfcauauggccuL96 | 1662 | asdGsgcdCadTaugadCaGfcacuccascsu | 2110 | AGUGGAGUGCUGUCAUAUGGCCU | 2558 |
| AD-1700248.1 | asgsugcuguCfAfUfauggccuggguL96 | 1663 | asdCscadGgdCcauadTgAfcagcacuscsc | 2111 | GGAGUGCUGUCAUAUGGCCUGGG | 2559 |
| AD-1700249.1 | gsusgcugucAfUfAfuggccuggguL96 | 1664 | asdCsccdAgdGccaudAuGfacagcacsusc | 2112 | GAGUGCUGUCAUAUGGCCUGGGA | 2560 |
| AD-1700250.1 | usgscugucaUfAfUfggccugggauL96 | 1665 | asdTsccdCadGgccadTaUfgacagcascsu | 2113 | AGUGCUGUCAUAUGGCCUGGGAC | 2561 |
| AD-1700252.1 | csusgucauaUfGfGfccugggacguL96 | 1666 | asdCsgudCcdCaggcdCaUfaugacagscsa | 2114 | UGCUGUCAUAUGGCCUGGGACGG | 2562 |
| AD-1700272.1 | asgsgcccaaGfCfCfAfcagcaaggauL96 | 1667 | asdTsccdTudGcugudGcUfugggccuscsu | 2115 | AGAGGCCCAAGCACAGCAAGGAC | 2563 |
| AD-1700279.1 | asgscacagcAfAfGfgacaucgccuL96 | 1668 | asdGsgcdGadTguccdTuGfcugugcususg | 2116 | CAAGCACAGCAAGGACAUCGCCC | 2564 |
| AD-1700280.1 | gscsacagcaAfGfGfacaucgcccuL96 | 1669 | asdGsggdCgdAugucdCuUfgcugugcsusu | 2117 | AAGCACAGCAAGGACAUCGCCCG | 2565 |
| AD-1700303.1 | uscsaccuuuGfAfCfguguacaaguL96 | 1670 | asdCsuudGudAcacgdTcAfaaggugasasu | 2118 | AUUCACCUUUGACGUGUACAAGC | 2566 |
| AD-1700309.1 | ususgacgugUfAfCfaagcaaaacuL96 | 1671 | asdGsuudTudGcuugdTaCfacgucaasasg | 2119 | CUUUGACGUGUACAAGCAAAACC | 2567 |
| AD-1700312.1 | ascsguguacAfAfGfcaaaacccuuL96 | 1672 | asdAsggdGudTuugcdTuGfuacacguscsa | 2120 | UGACGUGUACAAGCAAAACCCUC | 2568 |
| AD-1700314.1 | gsusguacaaGfCfAfaaacccucguL96 | 1673 | asdCsgadGgdGuuuudGcUfuguacacsgsu | 2121 | ACGUGUACAAGCAAAACCCUCGA | 2569 |
| AD-1700315.1 | usgsuacaagCfAfAfaacccucgauL96 | 1674 | asdTscgdAgdGguuudTgCfuuguacascsg | 2122 | CGUGUACAAGCAAAACCCUCGAG | 2570 |
| AD-1700316.1 | gsusacaagcAfAfAfacccucgaguL96 | 1675 | asdCsucdGadGgguudTuGfcuuguacsasc | 2123 | GUGUACAAGCAAAACCCUCGAGA | 2571 |
| AD-1700317.1 | usascaagcaAfAfAfcccucgagauL96 | 1676 | asdTscudCgdAgggudTuUfgcuuguascsa | 2124 | UGUACAAGCAAAACCCUCGAGAC | 2572 |
| AD-1700318.1 | ascsaagcaaAfAfCfccucgagacuL96 | 1677 | asdGsucdTcdGagggdTuUfugcuugusasc | 2125 | GUACAAGCAAAACCCUCGAGACC | 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 | csasaaacccUfCfGfagaccucuuuL96 | 1678 | asdAsagdAgdGucucdGaGfgguuuugscsu | 2126 | AGCAAAACCCUCGAGACCUCUUU | 2574 |
| AD-1700324.1 | asasaacccuCfGfAfgaccucuuuuL96 | 1679 | asdAsaadGadGgucudCgAfggguuuusgsc | 2127 | GCAAAACCCUCGAGACCUCUUUG | 2575 |
| AD-1700325.1 | asasacccucGfAfGfaccucuuuguL96 | 1680 | asdCsaadAgdAggucdTcGfaggguuususg | 2128 | CAAAACCCUCGAGACCUCUUUGG | 2576 |
| AD-1700327.1 | ascsccucgaGfAfCfcucuuuggcuL96 | 1681 | asdGsccdAadAgaggdTcUfcgagggususu | 2129 | AAACCCUCGAGACCUCUUUGGCA | 2577 |
| AD-1700328.1 | cscscucgagAfCfCfucuuuggcauL96 | 1682 | asdTsgcdCadAagagdGuCfucgagggsusu | 2130 | AACCCUCGAGACCUCUUUGGCAG | 2578 |
| AD-1700329.1 | cscsucgagaCfCfUfcuuuggcaguL96 | 1683 | asdCsugdCcdAaagadGgUfcucgaggsgsu | 2131 | ACCCUCGAGACCUCUUUGGCAGC | 2579 |
| AD-1700336.1 | ascscucuuuGfGfCfagccugaauuL96 | 1684 | asdAsuudCadGgcugdCcAfaagaggcscsu | 2132 | AGACCUCUUUGGCAGCCUGAAUG | 2580 |
| AD-1700339.1 | uscsuuuggcAfGfCfcugaaugucuL96 | 1685 | asdGsacdAudTcaggdCuGfccaaagasgsg | 2133 | CCUCUUUGGCAGCCUGAAUGUCA | 2581 |
| AD-1700344.1 | gsgscagccuGfAfAfugucaaagcuL96 | 1686 | asdGscudTudGacaudTcAfggcugccsasa | 2134 | UUGGCAGCCUGAAUGUCAAAGCC | 2582 |
| AD-1700350.1 | csusgaauguCfAfAfagccacauuuL96 | 1687 | asdAsaudGudGgcuudTgAfcauucagsgsc | 2135 | GCCUGAAUGUCAAAGCCACAUUC | 2583 |
| AD-1700352.1 | gsasaugucaAfAfGfccacauucuuL96 | 1688 | asdAsgadAudGuggcdTuUfgacauucsasg | 2136 | CUGAAUGUCAAAGCCACAUUCUA | 2584 |
| AD-1700353.1 | asasugucaaAfGfCfcacauucuauL96 | 1689 | asdTsagdAadTguggdCuUfugacauuscsa | 2137 | UGAAUGUCAAAGCCACAUUCUAC | 2585 |
| AD-1700354.1 | asusgucaaaGfCfCfacauucuacuL96 | 1690 | asdGsuadGadAugugdGcUfuugacaususc | 2138 | GAAUGUCAAAGCCACAUUCUACG | 2586 |
| AD-1700355.1 | usgsucaaagCfCfAfcauucuacguL96 | 1691 | asdCsgudAgdAaugudGgCfuuugacasusu | 2139 | AAUGUCAAAGCCACAUUCUACGG | 2587 |
| AD-1700356.1 | gsuscaaagcCfAfCfauucuacgguL96 | 1692 | asdCscgdTadGaaugdTgGfcuuugacsasu | 2140 | AUGUCAAAGCCACAUUCUACGGG | 2588 |
| AD-1700357.1 | uscsaaagccAfCfAfuucuacggguL96 | 1693 | asdCsccdGudAgaaudGuGfgcuuugascsa | 2141 | UGUCAAAGCCACAUUCUACGGGC | 2589 |
| AD-1700358.1 | csasaagccaCfAfUfucuacgggcuL96 | 1694 | asdGsccdCgdTagaadTgUfggcuuugsasc | 2142 | GUCAAAGCCACAUUCUACGGGCU | 2590 |
| AD-1700359.1 | asasagccacAfUfUfcuacgggcuuL96 | 1695 | asdAsgcdCcdGuagadAuGfuggcuuusgsa | 2143 | UCAAAGCCACAUUCUACGGGCUC | 2591 |
| AD-1700365.1 | ascsauucuaCfGfGfgcucuacucuL96 | 1696 | asdGsagdTadGagccdCgUfagaaugusgsg | 2144 | CCACAUUCUACGGGCUCUACUCU | 2592 |
| AD-1700366.1 | csasuucuacGfGfGfcucuacucuuL96 | 1697 | asdAsgadGudAgagcdCcGfuagaaugsusg | 2145 | CACAUUCUACGGGCUCUACUCUA | 2593 |
| AD-1700368.1 | ususcuacggGfCfUfcuacucuauuL96 | 1698 | asdAsuadGadGuagadGcCfcguagaasusg | 2146 | CAUUCUACGGGCUCUACUCUAUG | 2594 |
| AD-1700369.1 | uscsuacgggCfUfCfuacucuauguL96 | 1699 | asdCsaudAgdAguagdAgCfccguagasasu | 2147 | AUUCUACGGGCUCUACUCUAUGA | 2595 |
| AD-1700370.1 | csusacgggcUfCfUfacucuaugauL96 | 1700 | asdTscadTadGaguadGaGfcccguagsasa | 2148 | UUCUACGGGCUCUACUCUAUGAG | 2596 |
| AD-1700371.1 | usascgggcuCfUfAfcucuaugaguL96 | 1701 | asdCsucdAudAgagudAgAfgcccguasgsa | 2149 | UCUACGGGCUCUACUCUAUGAGU | 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 | ascsgggcucUfAfCfucuaugaguuL96 | 1702 | asdAscudCadTagagdTaGfagcccgusasg | 2150 | CUACGGGCUCUACUCUAUGAGUU | 2598 |
| AD-1700374.1 | gsgsgcucuaCfUfCfuaugaguuguL96 | 1703 | asdCsaadCudCauagdAgUfagagcccsgsu | 2151 | ACGGGCUCUACUCUAUGAGUUGU | 2599 |
| AD-1700375.1 | gsgscucuacUfCfUfaugaguuguuL96 | 1704 | asdAscadAcdTcauadGaGfuagagcccsg | 2152 | CGGGCUCUACUCUAUGAGUUGUG | 2600 |
| AD-1700376.1 | gscsucuacuCfUfAfugaguuguguL96 | 1705 | asdCsacdAadCucaudAgAfguagagcscsc | 2153 | GGGCUCUACUCUAUGAGUUGUGA | 2601 |
| AD-1700378.1 | uscsuacucuAfUfGfaguugugacuL96 | 1706 | asdGsucdAcdAacucdAuAfgaguagagsgsc | 2154 | GCUCUACUCUAUGAGUUGUGACU | 2602 |
| AD-1700383.1 | uscsuaugagUfUfGfugacuuucauL96 | 1707 | asdTsgadAadGucacdAaCfucauagagsgsu | 2155 | ACUCUAUGAGUUGUGACUUUCAA | 2603 |
| AD-1700384.1 | csusaugaguUfGfUfgacuuucaauL96 | 1708 | asdTsugdAadAgucadCaAfcucauagsasg | 2156 | CUCUAUGAGUUGUGACUUUCAAG | 2604 |
| AD-1700385.1 | usasugaguuGfUfGfacuuucaaguL96 | 1709 | asdCsuudGadAagucdAcAfacucauasgsa | 2157 | UCUAUGAGUUGUGACUUUCAAGG | 2605 |
| AD-1700386.1 | asusgaguugUfGfAfcuuucaagguL96 | 1710 | asdCscudTgdAaagudCaCfaacucausasg | 2158 | CUAUGAGUUGUGACUUUCAAGGA | 2606 |
| AD-1700387.1 | usgsaguuguGfAfCfuuucaaggauL96 | 1711 | asdTsccdTudGaaagdTcAfcaacucasusa | 2159 | UAUGAGUUGUGACUUUCAAGGAC | 2607 |
| AD-1700388.1 | gsasguugugAfCfUfuucaaggacuL96 | 1712 | asdGsucdCudTgaaadGuCfacaacucsasu | 2160 | AUGAGUUGUGACUUUCAAGGACU | 2608 |
| AD-1700408.1 | ususggcccaAfAfGfaaaguacucuL96 | 1713 | asdGsagdTadCuuucdTuUfgggccaasgsu | 2161 | ACUUGGCCCAAAGAAAGUACUCA | 2609 |
| AD-1700410.1 | gsgscccaaaGfAfAfaguacucaguL96 | 1714 | asdCsugdAgdTacuudTcUfuugggccsasa | 2162 | UUGGCCCAAAGAAAGUACUCAGG | 2610 |
| AD-1700411.1 | gscsccaaagAfAfAfguacucagguL96 | 1715 | asdCscudGadGuacudTuCfuuugggcscsa | 2163 | UGGCCCAAAGAAAGUACUCAGGG | 2611 |
| AD-1700413.1 | cscsaaagaaAfGfUfacucagggauL96 | 1716 | asdTsccdCudGaguadCuUfucuuuggsgsc | 2164 | GCCCAAAGAAAGUACUCAGGGAG | 2612 |
| AD-1700414.1 | csasaagaaaGfUfAfcucagggaguL96 | 1717 | asdCsucdCcdTgagudAcUfuucuuugsgsg | 2165 | CCCAAAGAAAGUACUCAGGGAGC | 2613 |
| AD-1700422.1 | gsusacucagGfGfAfgcuccuucguL96 | 1718 | asdCsgadAgdGagcudCcCfugaguacsusu | 2166 | AAGUACUCAGGGAGCUCCUUCGU | 2614 |
| AD-1700423.1 | usascucaggGfAfGfcuccuucguuL96 | 1719 | asdAscgdAadGgagcdTcCfcugaguascsu | 2167 | AGUACUCAGGGAGCUCCUUCGUU | 2615 |
| AD-1700424.1 | ascsucagggAfGfCfuccuucguuuL96 | 1720 | asdAsacdGadAggagdCuCfccugagusasc | 2168 | GUACUCAGGGAGCUCCUUCGUUG | 2616 |
| AD-1700425.1 | csuscagggaGfCfUfccuucguuguL96 | 1721 | asdCsaadCgdAaggadGcUfcccugagsusa | 2169 | UACUCAGGGAGCUCCUUCGUUGG | 2617 |
| AD-1700426.1 | uscsagggagCfUfCfcuucguugguL96 | 1722 | asdCscadAcdGaaggdAgCfucccugasgsu | 2170 | ACUCAGGGAGCUCCUUCGUUGGA | 2618 |
| AD-1700427.1 | csasgggagcUfCfCfuucguuggauL96 | 1723 | asdTsccdAadCgaagdGaGfcucccugsasg | 2171 | CUCAGGGAGCUCCUUCGUUGGAC | 2619 |
| AD-1700428.1 | asgsggagcuCfCfUfucguuggacuL96 | 1724 | asdGsucdCadAcgaadGgAfgcucccusgsa | 2172 | UCAGGGAGCUCCUUCGUUGGACC | 2620 |
| AD-1700439.1 | uscsguuggaCfCfUfccacacugcuL96 | 1725 | asdGscadGudGuggadGgUfccaacgasasg | 2173 | CUUCGUUGGACCUCCACACUGCU | 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 | csasaggccuGfGfGfccauauguuuL96 | 1726 | asdAsacdAudAuggcdCcAfggccuugscsa | 2174 | UGCAAGGCCUGGGCCAUAUGUUG | 2622 |
| AD-1700462.1 | asasggccugGfGfCfcauauguuguL96 | 1727 | asdCsaadCadTauggdCcCfaggccuusgsc | 2175 | GCAAGGCCUGGGCCAUAUGUUGC | 2623 |
| AD-1700464.1 | gsgsccugggCfCfAfuauguugcuuL96 | 1728 | asdAsgcdAadCauaudGgCfccaggccsusu | 2176 | AAGGCCUGGGCCAUAUGUUGCUG | 2624 |
| AD-1700465.1 | gscscugggcCfAfUfauguugcuguL96 | 1729 | asdCsagdCadAcauadTgGfcccaggcscsu | 2177 | AGGCCUGGGCCAUAUGUUGCUGG | 2625 |
| AD-1700468.1 | usgsggccauAfUfGfuugcugggauL96 | 1730 | asdTsccdCadGcaacdAuAfuggcccasgsg | 2178 | CCUGGGCCAUAUGUUGCUGGGAA | 2626 |
| AD-1700473.1 | csasuauguuGfCfUfgggaauuucuL96 | 1731 | asdGsaadAudTcccadGcAfacauaugsgsc | 2179 | GCCAUAUGUUGCUGGGAAUUUCC | 2627 |
| AD-1700474.1 | asusauguugCfUfGfggaauuuccuL96 | 1732 | asdGsgadAadTucccdAgCfaacauausgsg | 2180 | CCAUAUGUUGCUGGGAAUUUCCU | 2628 |
| AD-1700476.1 | asusguugcuGfGfGfaauuuccucuL96 | 1733 | asdGsagdGadAauucdCcAfgcaacausasu | 2181 | AUAUGUUGCUGGGAAUUUCCUCC | 2629 |
| AD-1700477.1 | usgsuugcugGfGfAfauuuccuccuL96 | 1734 | asdGsgadGgdAaauudCcCfagcaacasusa | 2182 | UAUGUUGCUGGGAAUUUCCUCCA | 2630 |
| AD-1700479.1 | ususgcugggAfAfUfuuccuccacuL96 | 1735 | asdGsugdGadGgaaadTuCfccagcaascsa | 2183 | UGUUGCUGGGAAUUUCCUCCACC | 2631 |
| AD-1700482.1 | csusgggaauUfUfCfcuccacccuuL96 | 1736 | asdAsggdGudGgaggdAaAfuucccagscsa | 2184 | UGCUGGGAAUUUCCUCCACCCUU | 2632 |
| AD-1700485.1 | gsgsaauuucCfUfCfcacccuucguL96 | 1737 | asdCsgadAgdGguggdAgGfaaauuccscsa | 2185 | UGGGAAUUUCCUCCACCCUUCGU | 2633 |
| AD-1700486.1 | gsasauuuccUfCfCfacccuucguuL96 | 1738 | asdAscgdAadGggugdGaGfgaaauucscsc | 2186 | GGGAAUUUCCUCCACCCUUCGUC | 2634 |
| AD-1700487.1 | asasuuuccuCfCfAfcccuucgucuL96 | 1739 | asdGsacdGadAgggudGgAfggaaauuscsc | 2187 | GGAAUUUCCUCCACCCUUCGUCA | 2635 |
| AD-1700488.1 | asusuuccucCfAfCfccuucgucauL96 | 1740 | asdTsgadCgdAagggdTgGfaggaaaususc | 2188 | GAAUUUCCUCCACCCUUCGUCAU | 2636 |
| AD-1700489.1 | ususuccuccAfCfCfcuucgucauuL96 | 1741 | asdAsugdAcdGaaggdGuGfgaggaaasusu | 2189 | AAUUUCCUCCACCCUUCGUCAUG | 2637 |
| AD-1700490.1 | ususccuccaCfCfCfucgucauguL96 | 1742 | asdCsaudGadCgaagdGgUfggaggaasasu | 2190 | AUUUCCUCCACCCUUCGUCAUGC | 2638 |
| AD-1700517.1 | asasgggccgCfCfUfccauuccuauL96 | 1743 | asdTsagdGadAuggadGgCfggcccuuscsu | 2191 | AGAAGGGCCGCCUCCAUUCCUAC | 2639 |
| AD-1700519.1 | gsgsgccgccUfCfCfauuccuacuuL96 | 1744 | asdAsgudAgdGaaugdGaGfgcggcccsusu | 2192 | AAGGGCCGCCUCCAUUCCUACUA | 2640 |
| AD-1700520.1 | gsgsccgccuCfCfAfuuccuacuauL96 | 1745 | asdTsagdTadGgaaudGgAfggcggccscsu | 2193 | AGGGCCGCCUCCAUUCCUACUAA | 2641 |
| AD-1700522.1 | cscsgccuccAfUfUfccuacuaaguL96 | 1746 | asdCsuudAgdTaggadAuGfgaggcggscsc | 2194 | GGCCGCCUCCAUUCCUACUAAGG | 2642 |
| AD-1700542.1 | cscscacugcAfAfAfgacuaugacuL96 | 1747 | asdGsucdAudAgucudTuGfcaguggsgsusc | 2195 | GACCCACUGCAAAGACUAUGACA | 2643 |
| AD-1700544.1 | csascugcaaAfGfAfcuaugacaguL96 | 1748 | asdCsugdTcdAuagudCuUfugcagugsgsg | 2196 | CCCACUGCAAAGACUAUGACAGC | 2644 |
| AD-1700550.1 | asasagacuaUfGfAfcagcaucaauL96 | 1749 | asdTsugdAudGcugudCaUfagucuuusgsc | 2197 | GCAAAGACUAUGACAGCAUCAAA | 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 | asdAsaudTudGaugcdTgUfcauagucsusu | 2198 | AAGACUAUGACAGCAUCAAAUUU | 2646 |
| AD-1700554.1 | ascsuaugacAfGfCfaucaaauuuuL96 | 1751 | asdAsaadTudTgaugdCuGfucauaguscsu | 2199 | AGACUAUGACAGCAUCAAAUUUC | 2647 |
| AD-1700555.1 | csusaugacaGfCfAfucaaauuucuL96 | 1752 | asdGsaadAudTugaudGcUfgucauagsusc | 2200 | GACUAUGACAGCAUCAAAUUUCA | 2648 |
| AD-1700556.1 | usasugacagCfAfUfcaaauuucauL96 | 1753 | asdTsgadAadTuugadTgCfugucauasgsu | 2201 | ACUAUGACAGCAUCAAAUUUCAG | 2649 |
| AD-1700557.1 | asusgacagcAfUfCfaaauuucaguL96 | 1754 | asdCsugdAadAuuugdAuGfcugucausasg | 2202 | CUAUGACAGCAUCAAAUUUCAGG | 2650 |
| AD-1700558.1 | usgsacagcaUfCfAfaauuucagguL96 | 1755 | asdCscudGadAauuudGaUfgcugucasusa | 2203 | UAUGACAGCAUCAAAUUUCAGGA | 2651 |
| AD-1700560.1 | ascsagcaucAfAfAfuuucaggacuL96 | 1756 | asdGsucdCudGaaaudTuGfaugcuguscsa | 2204 | UGACAGCAUCAAAUUUCAGGACC | 2652 |
| AD-1700563.1 | gscsaucaaaUfUfUfcaggaccuguL96 | 1757 | asdCsagdGudCcugadAaUfuugaugcsusg | 2205 | CAGCAUCAAAUUUCAGGACCUGC | 2653 |
| AD-1700566.1 | uscsaaauuuCfAfGfgaccugcaguL96 | 1758 | asdCsugdCadGguccdTgAfaauuugasusg | 2206 | CAUCAAAUUUCAGGACCUGCAGA | 2654 |
| AD-1700573.1 | uscsaggaccUfGfCfagacaguacuL96 | 1759 | asdGsuadCudGucugdCaGfguccugasasa | 2207 | UUUCAGGACCUGCAGACAGUACA | 2655 |
| AD-1700575.1 | asgsgaccugCfAfGfacaguacaguL96 | 1760 | asdCsugdTadCugucdTgCfagguccusgsa | 2208 | UCAGGACCUGCAGACAGUACAGG | 2656 |
| AD-1700576.1 | gsgsaccugcAfGfAfcaguacagguL96 | 1761 | asdCscudGudAcugudCuGfcagguccsusg | 2209 | CAGGACCUGCAGACAGUACAGGC | 2657 |
| AD-1700578.1 | ascscugcagAfCfAfguacaggcuuL96 | 1762 | asdAsgcdCudGuacudGuCfugcaggucscsc | 2210 | GGACCUGCAGACAGUACAGGCUA | 2658 |
| AD-1700580.1 | csusgcagacAfGfUfacaggcuaguL96 | 1763 | asdCsuadGcdCuguadCuGfucugcagsgsu | 2211 | ACCUGCAGACAGUACAGGCUAGA | 2659 |
| AD-1700582.1 | gscsagacagUfAfCfaggcuagauuL96 | 1764 | asdAsucdTadGccugdTaCfugucugcsasg | 2212 | CUGCAGACAGUACAGGCUAGAUA | 2660 |
| AD-1700623.1 | ascsgccucaCfAfUfccaagucuuL96 | 1765 | asdAsgadCudTgggadTgUfgaggcgususa | 2213 | UAACGCCUCACAUCCCAAGUCUA | 2661 |
| AD-1700670.1 | ususccuagcCfAfCfcuuucuggcuL96 | 1766 | asdGsccdAgdAaaggdTgGfcuaggaasasg | 2214 | CUUUCCUAGCCACCUUUCUGGCC | 2662 |
| AD-1700671.1 | uscscuagccAfCfCfuuucuggccuL96 | 1767 | asdGsgcdCadGaaagdGuGfgcuaggasasa | 2215 | UUUCCUAGCCACCUUUCUGGCCU | 2663 |
| AD-1700680.1 | cscsuuucugGfCfCfucccacuuguL96 | 1768 | asdCsaadGudGggagdGcCfagaaaggsusg | 2216 | CACCUUUCUGGCCUCCCACUUGC | 2664 |
| AD-1700681.1 | csusuucuggCfCfUfcccacuugcuL96 | 1769 | asdGscadAgdTgggadGgCfcagaaagsgsu | 2217 | ACCUUUCUGGCCUCCCACUUGCC | 2665 |
| AD-1700684.1 | uscsuggccuCfCfCfacuugcccuuL96 | 1770 | asdAsggdGcdAagugdGgAfggccagasasa | 2218 | UUUCUGGCCUCCCACUUGCCCUG | 2666 |
| AD-1700710.1 | gsgscaucuuGfAfUfcccagccuguL96 | 1771 | asdCsagdGcdTgggadTcAfagaugccsusg | 2219 | CAGGCAUCUUGAUCCCAGCCUGA | 2667 |
| AD-1700736.1 | asasugccucCfUfGfucccuaaacuL96 | 1772 | asdGsuudTadGggacdAgGfaggcauusgsg | 2220 | CCAAUGCCUCCUGUCCCUAAACU | 2668 |
| AD-1700737.1 | asusgccuccUfGfUfcccuaaacuuL96 | 1773 | asdAsgudTudAgggadCaGfgaggcaususg | 2221 | CAAUGCCUCCUGUCCCUAAACUC | 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 | usgsccuccuGfUfCfccuaaacucuL96 | 1774 | asdGsagdTudTagggdAcAfggaggcasusu | 2222 | AAUGCCUCCUGUCCCUAAACUCC | 2670 |
| AD-1700780.1 | csasaacuaaCfAfAfaaacauuucuL96 | 1775 | asdGsaadAudGuuuudTgUfuaguuugsasg | 2223 | CUCAAACUAACAAAAACAUUUCC | 2671 |
| AD-1700781.1 | asasacuaacAfAfAfaacauuuccuL96 | 1776 | asdGsgadAadTguuudTuGfuuaguuusgsa | 2224 | UCAAACUAACAAAAACAUUUCCA | 2672 |
| AD-1700782.1 | asascuaacaAfAfAfacauuuccauL96 | 1777 | asdTsggdAadAuguudTuUfguuaguususg | 2225 | CAAACUAACAAAAACAUUUCCAA | 2673 |
| AD-1700791.1 | gsusccugGfgCfUfGfagaucccaguL96 | 1778 | asCfsugdGg(Agn)ucucagCfcCfaggacsasa | 2226 | GGAAGGAAAGAGCAGAUCCCAGG | 2674 |
| AD-1700793.1 | usgsagauCfcCfAfGfguuuguaacuL96 | 1779 | asGfsuudAc(Agn)aaccugGfgAfucucasgsc | 2227 | GAGCAGAUCCCAGGUUUGUAACA | 2675 |
| AD-1700794.1 | gsasgaucCfcAfGfGfuuuguaacauL96 | 1780 | asUfsgudTa(Cgn)aaaccuGfgGfaucucsasg | 2228 | AGCAGAUCCCAGGUUUGUAACAG | 2676 |
| AD-1700795.1 | gsasucccAfgGfUfUfuguaacagauL96 | 1781 | asUfscudGu(Tgn)acaaacCfuGfggaucsusc | 2229 | CAGAUCCCAGGUUUGUAACAGAA | 2677 |
| AD-1700796.1 | cscsagguUfuGfUfAfacagaaaacuL96 | 1782 | asGfsuudTu(Cgn)uguuacAfaAfccuggsgsa | 2230 | UCCCAGGUUUGUAACAGAAAACA | 2678 |
| AD-1700797.1 | csasgguuUfgUfAfAfcagaaaacauL96 | 1783 | asUfsgudTu(Tgn)cuguuaCfaAfaccugsgsg | 2231 | CCCAGGUUUGUAACAGAAAACAC | 2679 |
| AD-1700798.1 | asgsguuuGfuAfAfCfagaaaacacuL96 | 1784 | asGfsugdTu(Tgn)ucuguuAfcAfaaccusgsg | 2232 | CCAGGUUUGUAACAGAAAACACC | 2680 |
| AD-1700799.1 | gsgsuuugUfaAfCfAfgaaaacaccuL96 | 1785 | asGfsgudGu(Tgn)uucuguUfaCfaaaccsusg | 2233 | CAGGUUUGUAACAGAAAACACCA | 2681 |
| AD-1700800.1 | usasacagAfaAfAfCfaccacuaaauL96 | 1786 | asUfsuudAg(Tgn)gguguuUfuCfuguuascsa | 2234 | UGUAACAGAAAACACCACUAAAG | 2682 |
| AD-1700801.1 | csasgaaaAfcAfCfCfcfacuaaagccuL96 | 1787 | asGfsgcdTu(Tgn)agugguGfuUfuucugsusu | 2235 | AACAGAAAACACCACUAAAGCCC | 2683 |
| AD-1700802.1 | csascaggAfgAfGfAfaccacccaguL96 | 1788 | asCfsugdGg(Tgn)gguucuCfuCfcugugscsu | 2236 | AGCACAGGAGAGAACCACCCAGC | 2684 |
| AD-1700804.1 | gsasaccaCfcCfAfGfcccagaaguuL96 | 1789 | asAfscudTc(Tgn)gggcugGfgUfgguucsusc | 2237 | GAGAACCACCCAGCCCAGAAGUU | 2685 |
| AD-1700805.1 | asasccacCfcAfGfCfccagaaguuuL96 | 1790 | asAfsacdTu(Cgn)ugggcuGfgGfugguuscsu | 2238 | AGAACCACCCAGCCCAGAAGUUC | 2686 |
| AD-1700806.1 | ascscaccCfaGfCfCfcagaaguucuL96 | 1791 | asGfsaadCu(Tgn)cugggcUfgGfguggususc | 2239 | GAACCACCCAGCCCAGAAGUUCC | 2687 |
| AD-1700807.1 | cscsacccAfgCfCfCfagaaguuccuL96 | 1792 | asGfsgadAc(Tgn)ucugggCfuGfgguggsusu | 2240 | AACCACCCAGCCCAGAAGUUCCA | 2688 |
| AD-1700808.1 | csascccaGfcCfCfAfgaaguuccauL96 | 1793 | asUfsggdAa(Cgn)uucuggGfcUfgggugsgsu | 2241 | ACCACCCAGCCCAGAAGUUCCAG | 2689 |
| AD-1700809.1 | cscsagaaGfuUfCfCffagggaaggauL96 | 1794 | asUfsccdTu(Cgn)ccuggaAfcUfucuggsgsc | 2242 | GCCCAGAAGUUCCAGGGAAGGAA | 2690 |
| AD-1700810.1 | csasgaagUfuCfCfAffgggaaggaauL96 | 1795 | asUfsucdCu(Tgn)cccuggAfaCfuucugsgsg | 2243 | CCCAGAAGUUCCAGGGAAGGAAC | 2691 |
| AD-1700811.1 | asgsaaguUfcCfAfGffggaaggaacuL96 | 1796 | asGfsuudCc(Tgn)ucccugGfaAfcuucusgsg | 2244 | CCAGAAGUUCCAGGGAAGGAACU | 2692 |
| AD-1700812.1 | gsasaguuCfcAfGfGffgaaggaacuuL96 | 1797 | asAfsgudTc(Cgn)uucccuGfgAfacuucsusg | 2245 | CAGAAGUUCCAGGGAAGGAACUC | 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 | asasguucCfaGfGfG faaggaacucuL96 | 1798 | asGfsagdTu(Cgn)cuuccc UfgGfaacuuscsu | 2246 | AGAAGUUCCAGGGA AGGAACUCU | 2694 |
| AD-1700814.1 | asgsuuccAfgGfGfA faggaacucuuL96 | 1799 | asAfsgadGu(Tgn)ccuucc CfuGfgaacususc | 2247 | GAAGUUCCAGGGAA GGAACUCUC | 2695 |
| AD-1700815.1 | gsusuccaGfgGfAfA fggaacucucuL96 | 1800 | asGfsagdAg(Tgn)uccuuc CfcUfggaacsusu | 2248 | AAGUUCCAGGGAAG GAACUCUCC | 2696 |
| AD-1700816.1 | gsusccacCfaUfGfG faguaccucuuL96 | 1801 | asAfsgadGg(Tgn)acucca UfgGfuggacscsg | 2249 | CGGUCCACCAUGGA GUACCUCUC | 2697 |
| AD-1700817.1 | csasccauGfgAfGfU faccucucaguL96 | 1802 | asCfsugdAg(Agn)gguacu CfcAfuggugsgsa | 2250 | UCCACCAUGGAGUA CCUCUCAGC | 2698 |
| AD-1700819.1 | cscsauggAfgUfAfC fcucucagcuuL96 | 1803 | asAfsgcdTg(Agn)gaggua CfuCfcauggsusg | 2251 | CACCAUGGAGUACC UCUCAGCUC | 2699 |
| AD-1700821.1 | asusggagUfaCfCfU fcucagcucuuL96 | 1804 | asAfsgadGc(Tgn)gagagg UfaCfuccausgsg | 2252 | CCAUGGAGUACCUC UCAGCUCUG | 2700 |
| AD-1700822.1 | ususacucAfgGfUfC faguaucuaauL96 | 1805 | asUfsuadGa(Tgn)acugac CfuGfaguaasgsu | 2253 | ACUUACUCAGGUCA GUAUCUAAU | 2701 |
| AD-1700824.1 | uscsaguaUfcUfAfA fuauaagcucuL96 | 1806 | asGfsagdCu(Tgn)auauua GfaUfacugascsc | 2254 | GGUCAGUAUCUAAU AUAAGCUCG | 2702 |
| AD-1700825.1 | csasguauCfuAfAfU fauaagcucguL96 | 1807 | asCfsgadGc(Tgn)uauauu AfgAfuacugsasc | 2255 | GUCAGUAUCUAAUA UAAGCUCGG | 2703 |
| AD-1700826.1 | asgsuaucUfaAfUfA fuaagcucgguL96 | 1808 | asCfscgdAg(Cgn)uuauau UfaGfauacusgsa | 2256 | UCAGUAUCUAAUAU AAGCUCGGA | 2704 |
| AD-1700828.1 | uscsuaauAfuAfAfG fcucggaguuuL96 | 1809 | asAfsacdTc(Cgn)gagcuu AfuAfuuagasusa | 2257 | UAUCUAAUAUAAGC UCGGAGUUU | 2705 |
| AD-1700829.1 | csusaauaUfaAfGfC fucggaguuuuL96 | 1810 | asAfsaadCu(Cgn)cgagcu UfaUfauuagsasu | 2258 | AUCUAAUAUAAGCU CGGAGUUUG | 2706 |
| AD-1700830.1 | usasagcuCfgGfAfG fuuuggacgguL96 | 1811 | asCfscgdTc(Cgn)aaacucC fgAfgcuuasusa | 2259 | UAUAAGCUCGGAGU UUGGACGGA | 2707 |
| AD-1700831.1 | asasgcucGfgAfGfU fuuggacggauL96 | 1812 | asUfscdGu(Cgn)caaacu CfcGfagcuusasu | 2260 | AUAAGCUCGGAGUU UGGACGGAG | 2708 |
| AD-1700832.1 | gsgsaguuUfgGfAf CfggagggucuuL96 | 1813 | asAfsgadCc(Cgn)uccguc CfaAfacuccsgsa | 2261 | UCGGAGUUUGGACG GAGGGUCUG | 2709 |
| AD-1700833.1 | usgsgacgGfaGfGfG fucuggaccuuL96 | 1814 | asAfsggdTc(Cgn)agaccc UfcCfguccasasa | 2262 | UUUGGACGGAGGGU CUGGACCUC | 2710 |
| AD-1700834.1 | asgscgacCfuUfUfC fcgugucuguuL96 | 1815 | asAfscadGa(Cgn)acggaa AfgGfucgcusgsg | 2263 | CCAGCGACCUUUCC GUGUCUGUG | 2711 |
| AD-1700837.1 | csasagcgGfaCfCfA fuccggaaaguL96 | 1816 | asCfsuudTc(Cgn)ggaugg UfcCfgcuugsusg | 2264 | CACAAGCGGACCAU CCGGAAAGG | 2712 |
| AD-1700838.1 | csasuccgGfaAfAfG fgccugacaguL96 | 1817 | asCfsugdTc(Agn)ggccuu UfcCfggaugsgsu | 2265 | ACCAUCCGGAAAGG CCUGACAGC | 2713 |
| AD-1700839.1 | asgsgagcUfgCfUfA fgccaaagcauL96 | 1818 | asUfsgcdTu(Tgn)ggcuag CfaGfcuccusgsg | 2266 | CCAGGAGCUGCUAG CCAAAGCAU | 2714 |
| AD-1700840.1 | gsgsagcuGfcUfAfG fccaaagcauuL96 | 1819 | asAfsugdCu(Tgn)uggcua GfcAfgcuccsusg | 2267 | CAGGAGCUGCUAGC CAAAGCAUU | 2715 |
| AD-1700841.1 | gsasgcugCfuAfGfC fcaaagcauuuL96 | 1820 | asAfsaudGc(Tgn)uuggcu AfgCfagcucscsu | 2268 | AGGAGCUGCUAGCC AAAGCAUUG | 2716 |
| AD-1700842.1 | asgscugcUfaGfCfC faaagcauuguL96 | 1821 | asCfsaadTg(Cgn)uuuggc UfaGfcagcuscsc | 2269 | GGAGCUGCUAGCCA AAGCAUUGG | 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 fcccuacugcuL96 | 1824 | asGfscadGu(Agn)gggucu CfcAfaugcususu | 2272 | AAAGCAUUGGAGAC CCUACUGCU | 2720 |
| AD-1700846.1 | gscsauugGfaGfAfC fccuacugcuuL96 | 1825 | asAfsgcdAg(Tgn)aggguc UfcCfaaugcsusu | 2273 | AAGCAUUGGAGACC CUACUGCUG | 2721 |
| AD-1700848.1 | asusuggaGfaCfCfC fuacugcugauL96 | 1826 | asUfscadGc(Agn)guaggg UfcUfccaausgsc | 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 | ascsccuaCfuGfCfU fgaauggaguuL96 | 1829 | asAfscudCc(Agn)uucagc AfgUfagggucsu | 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)cauuca GfcAfguaggsgsu | 2279 | ACCCUACUGCUGAA UGGAGUGCU | 2727 |
| AD-1700855.1 | csusacugCfuGfAfA fuggagugcuuL96 | 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 fugcuaacccuL96 | 1834 | asGfsggdTu(Agn)gcacuc CfaUfucagcsasg | 2282 | CUGCUGAAUGGAGU GCUAACCCU | 2730 |
| AD-1700859.1 | csusgaauGfgAfGfU fgcuaacccuuL96 | 1835 | asAfsggdGu(Tgn)agcacu CfcAfuucagscsa | 2283 | UGCUGAAUGGAGUG CUAACCCUG | 2731 |
| AD-1700860.1 | usgsaaugGfaGfUfG fcuaacccuguL96 | 1836 | asCfsagdGg(Tgn)uagcac UfcCfauucasgsc | 2284 | GCUGAAUGGAGUGC UAACCCUGG | 2732 |
| AD-1700863.1 | usgsgaguGfcUfAf Afcccuggugcu L96 | 1837 | asGfscadCc(Agn)ggguua GfcAfcuccasusu | 2285 | AAUGGAGUGCUAAC CCUGGUGCU | 2733 |
| AD-1700864.1 | gsgsagugCfuAfAfC fccuggugcuuL96 | 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 | asascccuGfgUfGfC fuagaggaggu L96 | 1840 | asCfscudCc(Tgn)cuagca CfcAfggguusasg | 2288 | CUAACCCUGGUGCU AGAGGAGGA | 2736 |
| AD-1700868.1 | cscscuggUfgCfUfA fgaggaggauuL96 | 1841 | asAfsucdCu(Cgn)cucuag CfaCfcagggsusu | 2289 | AACCCUGGUGCUAG AGGAGGAUG | 2737 |
| AD-1700869.1 | gsgsugcuAfgAfGf Gfaggauggaau L96 | 1842 | asUfsucdCa(Tgn)ccuccu CfuAfgcaccsasg | 2290 | CUGGUGCUAGAGGA GGAUGGAAC | 2738 |
| AD-1700870.1 | gsusgcuaGfaGfGfA fggauggaacuL96 | 1843 | asGfsuudCc(Agn)uccucc UfcUfagcacscsa | 2291 | UGGUGCUAGAGGAG GAUGGAACU | 2739 |
| AD-1700871.1 | csusagagGfaGfGfA fuggaacugcuL96 | 1844 | asGfscadGu(Tgn)ccaucc UfcCfucuagscsa | 2292 | UGCUAGAGGAGGAU GGAACUGCA | 2740 |
| AD-1700872.1 | gsasggauGfgAfAfC fugcaguggauL96 | 1845 | asUfsccdAc(Tgn)gcaguu CfcAfuccucscsu | 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 | asAfscudGu(Cgn)cacugcAfgUfuccauscsc | 2296 | GGAUGGAACUGCAGUGGACAGUG | 2744 |
| AD-1700876.1 | usgsgaacUfgCfAfGfuggacaguguL96 | 1849 | asCfsacdTg(Tgn)ccacugCfaGfuuccasusc | 2297 | GAUGGAACUGCAGUGGACAGUGA | 2745 |
| AD-1700877.1 | gsasacugCfaGfUfGfgacagugaguL96 | 1850 | asCfsucdAc(Tgn)guccacUfgCfaguucscsa | 2298 | UGGAACUGCAGUGGACAGUGAGG | 2746 |
| AD-1700878.1 | ascsugcaGfuGfGfAfcagugaggauL96 | 1851 | asUfsccdTc(Agn)cugccAfcUfgcagususc | 2299 | GAACUGCAGUGGACAGUGAGGAC | 2747 |
| AD-1700879.1 | csusgcagUfgGfAfCfagugaggacuL96 | 1852 | asGfsucdCu(Cgn)acugucCfaCfugcagsusu | 2300 | AACUGCAGUGGACAGUGAGGACU | 2748 |
| AD-1700880.1 | usgscaguGfgAfCfAfgugaggacuuL96 | 1853 | asAfsgudCc(Tgn)cacuguCfcAfcugcasgsu | 2301 | ACUGCAGUGGACAGUGAGGACUU | 2749 |
| AD-1700881.1 | gscsagugGfaCfAfGfugaggacuuuL96 | 1854 | asAfsagdTc(Cgn)ucacugUfcCfacugcsasg | 2302 | CUGCAGUGGACAGUGAGGACUUC | 2750 |
| AD-1700882.1 | csasguggAfcAfGfUfgaggacuucuL96 | 1855 | asGfsaadGu(Cgn)cucacuGfuCfcacugcsca | 2303 | UGCAGUGGACAGUGAGGACUUCU | 2751 |
| AD-1700883.1 | asgsuggaCfaGfUfGfaggacuucuuL96 | 1856 | asAfsgadAg(Tgn)ccucacUfgUfccacusgsc | 2304 | GCAGUGGACAGUGAGGACUUCUU | 2752 |
| AD-1700885.1 | usgsgacaGfuGfAfGfgacuucuucuL96 | 1857 | asGfsaadGa(Agn)guccucAfcUfguccascsu | 2305 | AGUGGACAGUGAGGACUUCUUCC | 2753 |
| AD-1700887.1 | csasgugaGfgAfCfUfucuuccagcuL96 | 1858 | asGfscudGg(Agn)agaaguCfcUfcacugsusc | 2306 | GACAGUGAGGACUUCUUCCAGCU | 2754 |
| AD-1700888.1 | usgsaggaCfuUfCfUfuccagcugcuL96 | 1859 | asGfscadGc(Tgn)ggaagaAfgUfccucascsu | 2307 | AGUGAGGACUUCUUCCAGCUGCU | 2755 |
| AD-1700889.1 | gsasggacUfuCfUfUfccagcugcuuL96 | 1860 | asAfsgcdAg(Cgn)uggaagAfaGfuccucsasc | 2308 | GUGAGGACUUCUUCCAGCUGCUG | 2756 |
| AD-1700891.1 | gsgsacuuCfuUfCfCffagcugcugguL96 | 1861 | asCfscadGc(Agn)gcuggaAfgAfaguccsusc | 2309 | GAGGACUUCUUCCAGCUGCUGGA | 2757 |
| AD-1700892.1 | uscsuuccAfgCfUfGffcuggaggauuL96 | 1862 | asAfsucdCu(Cgn)cagcagCfuGfgaagasasg | 2310 | CUUCUUCCAGCUGCUGGAGGAUG | 2758 |
| AD-1700893.1 | csasgcugCfuGfGfAffggaugacacuL96 | 1863 | asGfsugdTc(Agn)uccuccAfgCfagcugsgsa | 2311 | UCCAGCUGCUGGAGGAUGACACG | 2759 |
| AD-1700894.1 | gscsugcuGfgAfGfGfaugacacguuL96 | 1864 | asAfscgdTg(Tgn)cauccuCfcAfgcagcsusg | 2312 | CAGCUGCUGGAGGAUGACACGUG | 2760 |
| AD-1700895.1 | csusggagGfaUfGfAfcacgugccuuL96 | 1865 | asAfsggdCa(Cgn)guguccaUfcCfuccagscsa | 2313 | UGCUGGAGGAUGACACGUGCCUG | 2761 |
| AD-1700897.1 | gsgsaugaCfaCfGfUfgccugaugguL96 | 1866 | asCfscadTc(Agn)ggcacgUfgUfcauccsusc | 2314 | GAGGAUGACACGUGCCUGAUGGU | 2762 |
| AD-1700898.1 | gsasugacAfcGfUfGfccugaugguuL96 | 1867 | asAfsccdAu(Cgn)aggcacGfuGfucaucscsu | 2315 | AGGAUGACACGUGCCUGAUGGUG | 2763 |
| AD-1700899.1 | gsascacgUfgCfCfUfgaugguguuuL96 | 1868 | asAfsacdAc(Cgn)aucaggCfaCfgugucsasu | 2316 | AUGACACGUGCCUGAUGGUGUUG | 2764 |
| AD-1700900.1 | gscsagucUfgGfUfCfagagcuggauL96 | 1869 | asUfsccdAg(Cgn)ucugacCfaGfacugcsasa | 2317 | UUGCAGUCUGGUCAGAGCUGGAG | 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 | asAfscadGc(Agn)cuccacUfcCfuuguasgsg | 2318 | CCUACAAGGAGUGGAGUGCUGUC | 2766 |
| AD-1700903.1 | ascsaaggAfgUfGfGfagugcugucuL96 | 1871 | asGfsacdAg(Cgn)acuccaCfuCfcuugusasg | 2319 | CUACAAGGAGUGGAGUGCUGUCA | 2767 |
| AD-1700904.1 | asasggagUfgGfAfGfugcugucauuL96 | 1872 | asAfsugdAc(Agn)gcacucCfaCfuccuusgsu | 2320 | ACAAGGAGUGGAGUGCUGUCAUA | 2768 |
| AD-1700905.1 | gsgsagugGfaGfUfGfcugucauauuL96 | 1873 | asAfsuadTg(Agn)cagcacUfcCfacuccsusu | 2321 | AAGGAGUGGAGUGCUGUCAUAUG | 2769 |
| AD-1700906.1 | usgsgaguGfcUfGfUfcauauggccuL96 | 1874 | asGfsgcdCa(Tgn)augacaGfcAfcuccascsu | 2322 | AGUGGAGUGCUGUCAUAUGGCCU | 2770 |
| AD-1700907.1 | gsgsagugCfuGfUfCfauauggccuuL96 | 1875 | asAfsggdCc(Agn)uaugacAfgCfacuccsasc | 2323 | GUGGAGUGCUGUCAUAUGGCCUG | 2771 |
| AD-1700908.1 | gsasgugcUfgUfCfAfuauggccuguL96 | 1876 | asCfsagdGc(Cgn)auaugaCfaGfcacucscsa | 2324 | UGGAGUGCUGUCAUAUGGCCUGG | 2772 |
| AD-1700909.1 | asgsugcuGfuCfAfUfauggccugguL96 | 1877 | asCfscadGg(Cgn)cauaugAfcAfgcacuscsc | 2325 | GGAGUGCUGUCAUAUGGCCUGGG | 2773 |
| AD-1700912.1 | gscsugucAfuAfUfGfgccugggacuL96 | 1878 | asGfsucdCc(Agn)ggccauAfuGfacagcsasc | 2326 | GUGCUGUCAUAUGGCCUGGGACG | 2774 |
| AD-1700913.1 | csusgucaUfaUfGfGffccugggacguL96 | 1879 | asCfsgudCc(Cgn)aggccaUfaUfgacagscsa | 2327 | UGCUGUCAUAUGGCCUGGGACGG | 2775 |
| AD-1700915.1 | csasagcaCfaGfCfAfaggacaucguL96 | 1880 | asCfsgadTg(Tgn)ccuugcUfgUfgcuugsgsg | 2328 | CCCAAGCACAGCAAGGACAUCGC | 2776 |
| AD-1700916.1 | asusucacCfuUfUfGfacguguacauL96 | 1881 | asUfsgudAc(Agn)cgucaaAfgGfugaauscsg | 2329 | CGAUUCACCUUUGACGUGUACAA | 2777 |
| AD-1700917.1 | ususcaccUfuUfGfAfcguguacaauL96 | 1882 | asUfsugdTa(Cgn)acgucaAfaGfgugaasusc | 2330 | GAUUCACCUUUGACGUGUACAAG | 2778 |
| AD-1700918.1 | uscsaccuUfuGfAfCfguguacaaguL96 | 1883 | asCfsuudGu(Agn)cacgucAfaAfggugasasu | 2331 | AUUCACCUUUGACGUGUACAAGC | 2779 |
| AD-1700919.1 | csasccuuUfgAfCfGfuguacaagcuL96 | 1884 | asGfscudTg(Tgn)acacguCfaAfaggugsasa | 2332 | UUCACCUUUGACGUGUACAAGCA | 2780 |
| AD-1700920.1 | cscsuuugAfcGfUfGfuacaagcaauL96 | 1885 | asUfsugdCu(Tgn)guacacGfuCfaaaggsusg | 2333 | CACCUUUGACGUGUACAAGCAAA | 2781 |
| AD-1700922.1 | usgsacguGfuAfCfAfagcaaaaccuL96 | 1886 | asGfsgudTu(Tgn)gcuuguAfcAfcgucasasa | 2334 | UUUGACGUGUACAAGCAAAACCC | 2782 |
| AD-1700923.1 | gsascgugUfaCfAfAfgcaaaacccuL96 | 1887 | asGfsggdTu(Tgn)ugcuugUfaCfacgucsasa | 2335 | UUGACGUGUACAAGCAAAACCCU | 2783 |
| AD-1700924.1 | ascsguguAfcAfAfGfcaaaacccuuL96 | 1888 | asAfsggdGu(Tgn)uugcuuGfuAfcacguscsa | 2336 | UGACGUGUACAAGCAAAACCCUC | 2784 |
| AD-1700925.1 | csgsuguaCfaAfGfCfaaaacccucuL96 | 1889 | asGfsagdGg(Tgn)uuugcuUfgUfacacgsusc | 2337 | GACGUGUACAAGCAAAACCCUCG | 2785 |
| AD-1700929.1 | csasagcaAfaAfCfCfcfcucgagaccuL96 | 1890 | asGfsgudCu(Cgn)gaggguUfuUfgcuugsusa | 2338 | UACAAGCAAAACCCUCGAGACCU | 2786 |
| AD-1700930.1 | asasgcaaAfaCfCfCfCfucgagaccuuL96 | 1891 | asAfsggdTc(Tgn)cgagggUfuUfugcuusgsu | 2339 | ACAAGCAAAACCCUCGAGACCUC | 2787 |
| AD-1700931.1 | asgscaaaAfcCfCfUfcgagaccucuL96 | 1892 | asGfsagdGu(Cgn)ucgaggGfuUfuugcususg | 2340 | CAAGCAAAACCCUCGAGACCUCU | 2788 |
| AD-1700934.1 | gsasgaccUfcUfUfUfggcagccuguL96 | 1893 | asCfsagdGc(Tgn)gccaaaGfaGfgucucsgsa | 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 | cscsucuuUfgGfCfAfgccugaauguL96 | 1894 | asCfsaudTc(Agn)ggcugcCfaAfagaggsusc | 2342 | GACCUCUUUGGCAGCCUGAAUGU | 2790 |
| AD-1700937.1 | csuscuuuGfgCfAfGfccugaauguuL96 | 1895 | asAfscadTu(Cgn)aggcugCfcAfaagagsgsu | 2343 | ACCUCUUUGGCAGCCUGAAUGUC | 2791 |
| AD-1700938.1 | csusuuggCfaGfCfCfugaaugucauL96 | 1896 | asUfsgadCa(Tgn)ucaggcUfgCfcaaagsasg | 2344 | CUCUUUGGCAGCCUGAAUGUCAA | 2792 |
| AD-1700939.1 | ususuggcAfgCfCfUfgaaugucaauL96 | 1897 | asUfsugdAc(Agn)uucaggCfuGfccaaasgsa | 2345 | UCUUUGGCAGCCUGAAUGUCAAA | 2793 |
| AD-1700940.1 | usgsgcagCfcUfGfAfaugucaaaguL96 | 1898 | asCfsuudTg(Agn)cauucaGfgCfugccasasa | 2346 | UUUGGCAGCCUGAAUGUCAAAGC | 2794 |
| AD-1700942.1 | gscsagccUfgAfAfUfgucaaagccuL96 | 1899 | asGfsgcdTu(Tgn)gacauuCfaGfgcugcscsa | 2347 | UGGCAGCCUGAAUGUCAAAGCCA | 2795 |
| AD-1700943.1 | csasgccuGfaAfUfGfucaaagccauL96 | 1900 | asUfsggdCu(Tgn)ugacauUfcAfggcugscsc | 2348 | GGCAGCCUGAAUGUCAAAGCCAC | 2796 |
| AD-1700944.1 | asgsccugAfaUfGfUfcaaagccacuL96 | 1901 | asGfsugdGc(Tgn)uugacaUfuCfaggcusgsc | 2349 | GCAGCCUGAAUGUCAAAGCCACA | 2797 |
| AD-1700947.1 | asusgucaAfaGfCfCffacauucuacuL96 | 1902 | asGfsuadGa(Agn)uguggcUfuUfgacaususc | 2350 | GAAUGUCAAAGCCACAUUCUACG | 2798 |
| AD-1700948.1 | asgsccacAfuUfCfUffacgggcucuuL96 | 1903 | asAfsgadGc(Cgn)cguagaAfuGfuggcususu | 2351 | AAAGCCACAUUCUACGGGCUCUA | 2799 |
| AD-1700949.1 | gscscacaUfuCfUfAffcgggcucuauL96 | 1904 | asUfsagdAg(Cgn)ccguagAfaUfguggcsusu | 2352 | AAGCCACAUUCUACGGGCUCUAC | 2800 |
| AD-1700950.1 | csascauuCfuAfCfGfggcucuacuuL96 | 1905 | asAfsgudAg(Agn)gcccguAfgAfaugugsgsc | 2353 | GCCACAUUCUACGGGCUCUACUC | 2801 |
| AD-1700952.1 | csasuucuAfcGfGfGfcucuacucuuL96 | 1906 | asAfsgadGu(Agn)gagcccGfuAfgaaugsusg | 2354 | CACAUUCUACGGGCUCUACUCUA | 2802 |
| AD-1700953.1 | asusucuaCfgGfGfCfucuacucuauL96 | 1907 | asUfsagdAg(Tgn)agagccCfgUfagaausgsu | 2355 | ACAUUCUACGGGCUCUACUCUAU | 2803 |
| AD-1700955.1 | uscsuacgGfgCfUfCfuacucuauguL96 | 1908 | asCfsaudAg(Agn)guagagCfcCfguagasasu | 2356 | AUUCUACGGGCUCUACUCUAUGA | 2804 |
| AD-1700957.1 | ascsgggcUfcUfAfCfucuaugaguuL96 | 1909 | asAfscudCa(Tgn)agaguaGfaGfcccgusasg | 2357 | CUACGGGCUCUACUCUAUGAGUU | 2805 |
| AD-1700958.1 | gsgsgcucUfaCfUfCfuaugaguuguL96 | 1910 | asCfsaadCu(Cgn)auagagUfaGfagcccsgsu | 2358 | ACGGGCUCUACUCUAUGAGUUGU | 2806 |
| AD-1700959.1 | gsgscucuAfcUfCfUfaugaguuguuL96 | 1911 | asAfscadAc(Tgn)cauagaGfuAfgagccscsg | 2359 | CGGGCUCUACUCUAUGAGUUGUG | 2807 |
| AD-1700960.1 | csusacucUfaUfGfAfguugugacuuL96 | 1912 | asAfsgudCa(Cgn)aacucaUfaGfaguagsasg | 2360 | CUCUACUCUAUGAGUUGUGACUU | 2808 |
| AD-1700961.1 | usascucuAfuGfAfGfuugugacuuuL96 | 1913 | asAfsagdTc(Agn)caacucAfuAfgaguasgsa | 2361 | UCUACUCUAUGAGUUGUGACUUU | 2809 |
| AD-1700962.1 | ascsucuaUfgAfGfUfugugacuuuuL96 | 1914 | asAfsaadGu(Cgn)acaacuCfaUfagagusasg | 2362 | CUACUCUAUGAGUUGUGACUUUC | 2810 |
| AD-1700963.1 | csuscuauGfaGfUfUfgugacuuucuL96 | 1915 | asGfsaadAg(Tgn)cacaacUfcAfuagagsusa | 2363 | UACUCUAUGAGUUGUGACUUUCA | 2811 |
| AD-1700965.1 | csusaugaGfuUfGfUfgacuuucaauL96 | 1916 | asUfsugdAa(Agn)gucacaAfcUfcauagsasg | 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 | asgsuuguGfaCfUfU fucaaggacuuL96 | 1918 | asAfsgudCc(Tgn)ugaaag UfcAfcaacuscsa | 2366 | UGAGUUGUGACUUU CAAGGACUU | 2814 |
| AD-1700969.1 | ususggccCfaAfAfG faaaguacucuL96 | 1919 | asGfsagdTa(Cgn)uuucuu UfgGfgccaasgsu | 2367 | ACUUGGCCCAAAGA AAGUACUCA | 2815 |
| AD-1700970.1 | usgsgcccAfaAfGfA faaguacucauL96 | 1920 | asUfsgadGu(Agn)cuuucu UfuGfggccasasg | 2368 | CUUGGCCCAAAGAA AGUACUCAG | 2816 |
| AD-1700971.1 | gsgscccaAfaGfAfA faguacucaguL96 | 1921 | asCfsugdAg(Tgn)acuuuc UfuUfgggccsasa | 2369 | UUGGCCCAAAGAAA GUACUCAGG | 2817 |
| AD-1700973.1 | cscscaaaGfaAfAfG fuacucaggguL96 | 1922 | asCfsccdTg(Agn)guacuu UfcUfuugggcsc | 2370 | GGCCCAAAGAAAGU ACUCAGGGA | 2818 |
| AD-1700975.1 | asasagaaAfgUfAfC fucagggagcuL96 | 1923 | asGfscudCc(Cgn)ugagua CfuUfucuuusgsg | 2371 | CCAAAGAAAGUACU CAGGGAGCU | 2819 |
| AD-1700976.1 | asasgaaaGfuAfCfU fcagggagcuuL96 | 1924 | asAfsgcdTc(Cgn)cugagu AfcUfuucuusug | 2372 | CAAAGAAAGUACUC AGGGAGCUC | 2820 |
| AD-1700977.1 | asgsaaagUfaCfUfC fagggagcucuL96 | 1925 | asGfsagdCu(Cgn)ccugag UfaCfuuucususu | 2373 | AAAGAAAGUACUCA GGGAGCUCC | 2821 |
| AD-1700978.1 | asgsuacuCfaGfGfG fagcuccuucuL96 | 1926 | asGfsaadGg(Agn)gcuccc UfgAfguacususu | 2374 | AAAGUACUCAGGGA GCUCCUUCG | 2822 |
| AD-1700980.1 | asgsggagCfuCfCfU fucguuggacuL96 | 1927 | asGfsucdCa(Agn)cgaagg AfgCfucccusgsa | 2375 | UCAGGGAGCUCCUU CGUUGGACC | 2823 |
| AD-1700981.1 | gsgsagcuCfcUfUfC fguuggaccuuL96 | 1928 | asAfsggdTc(Cgn)aacgaa GfgAfgcucscsu | 2376 | AGGGAGCUCCUUCG UUGGACCUC | 2824 |
| AD-1700982.1 | ususcguuGfgAfCfC fuccacacuguL96 | 1929 | asCfsagdTg(Tgn)ggaggu CfcAfacgaasgsg | 2377 | CCUUCGUUGGACCU CCACACUGC | 2825 |
| AD-1700984.1 | csgsuuggAfcCfUfC fcacacugcuuL96 | 1930 | asAfsgcdAg(Tgn)guggag GfuCfcaacgsasa | 2378 | UUCGUUGGACCUCC ACACUGCUG | 2826 |
| AD-1700985.1 | gsasccucCfaCfAfC fugcugcaaguL96 | 1931 | asCfsuudGc(Agn)gcagug UfgGfaggucscsa | 2379 | UGGACCUCCACACU GCUGCAAGG | 2827 |
| AD-1700986.1 | gsgsccugGfgCfCfA fuauguugcuuL96 | 1932 | asAfsgcdAa(Cgn)auaugg CfcCfaggccsusu | 2380 | AAGGCCUGGGCCAU AUGUUGCUG | 2828 |
| AD-1700988.1 | gsgsgccaUfaUfGfU fugcugggaauL96 | 1933 | asUfsucdCc(Agn)gcaaca UfaUfggcccsasg | 2381 | CUGGGCCAUAUGUU GCUGGGAAU | 2829 |
| AD-1700989.1 | gsgsccauAfuGfUfU fgcugggaauuL96 | 1934 | asAfsuudCc(Cgn)agcaac AfuAfuggccscsa | 2382 | UGGGCCAUAUGUUG CUGGGAAUU | 2830 |
| AD-1700990.1 | cscsauauGfuUfGfC fugggaauuuuL96 | 1935 | asAfsaadTu(Cgn)ccagca AfcAfuauggscsc | 2383 | GGCCAUAUGUUGCU GGGAAUUUC | 2831 |
| AD-1700991.1 | csasuaugUfuGfCfU fgggaauuucuL96 | 1936 | asGfsaadAu(Tgn)cccagc AfaCfauaugsgsc | 2384 | GCCAUAUGUUGCUG GGAAUUUCC | 2832 |
| AD-1700992.1 | usgsuugcUfgGfGf Afauuuccuccu L96 | 1937 | asGfsgadGg(Agn)aauucc CfaGfcaacasusa | 2385 | UAUGUUGCUGGGAA UUUCCUCCA | 2833 |
| AD-1700995.1 | usgsggaaUfuUfCfC fuccacccuuuL96 | 1938 | asAfsagdGg(Tgn)ggagga AfaUfucccasgsc | 2386 | GCUGGGAAUUUCCU CCACCCUUC | 2834 |
| AD-1700997.1 | ususccucCfaCfCfC fuucgucauguL96 | 1939 | asCfsaudGa(Cgn)gaaggg UfgGfaggaasasu | 2387 | AUUUCCUCCACCCU UCGUCAUGC | 2835 |
| AD-1700998.1 | asasgggcCfgCfCfU fccauuccuauL96 | 1940 | asUfsagdGa(Agn)uggagg CfgGfcccuuscsu | 2388 | AGAAGGGCCGCCUC CAUUCCUAC | 2836 |
| AD-1701001.1 | gscscgccUfcCfAfU fuccuacuaauL96 | 1941 | asUfsuadGu(Agn)ggaaug GfaGfgcggcscsc | 2389 | GGGCCGCCUCCAUU 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 | cscsgccuCfcAfUfUfcccuacuaaguL96 | 1942 | asCfsuudAg(Tgn)aggaauGfgAfggcggscsc | 2390 | GGCCGCCUCCAUUCCUACUAAGG | 2838 |
| AD-1701003.1 | cscscacuGfcAfAfAfgacuaugacuL96 | 1943 | asGfsucdAu(Agn)gucuuuGfcAfgugggsusc | 2391 | GACCCACUGCAAAGACUAUGACA | 2839 |
| AD-1701004.1 | cscsacugCfaAfAfGfacuaugacauL96 | 1944 | asUfsgudCa(Tgn)agucuuUfgCfaguggsgsu | 2392 | ACCCACUGCAAAGACUAUGACAG | 2840 |
| AD-1701005.1 | csascugcAfaAfGfAfcuaugacaguL96 | 1945 | asCfsugdTc(Agn)uagucuUfuGfcagugsgsg | 2393 | CCCACUGCAAAGACUAUGACAGC | 2841 |
| AD-1701007.1 | asasgacuAfuGfAfCfagcaucaaauL96 | 1946 | asUfsuudGa(Tgn)gcugucAfuAfgucuususg | 2394 | CAAAGACUAUGACAGCAUCAAAU | 2842 |
| AD-1701009.1 | gsascagcAfuCfAfAfauuucaggauL96 | 1947 | asUfsccdTg(Agn)aauuugAfuGfcugucsasu | 2395 | AUGACAGCAUCAAAUUUCAGGAC | 2843 |
| AD-1701011.1 | csasgcauCfaAfAfUfuucaggaccuL96 | 1948 | asGfsgudCc(Tgn)gaaauuUfgAfugcugsusc | 2396 | GACAGCAUCAAAUUUCAGGACCU | 2844 |
| AD-1701012.1 | asgscaucAfaAfUfUffucaggaccuuL96 | 1949 | asAfsggdTc(Cgn)ugaaauUfuGfaugcusgsu | 2397 | ACAGCAUCAAAUUUCAGGACCUG | 2845 |
| AD-1701013.1 | gscsaucaAfaUfUfUfcaggaccuguL96 | 1950 | asCfsagdGu(Cgn)cugaaaUfuUfgaugcsusg | 2398 | CAGCAUCAAAUUUCAGGACCUGC | 2846 |
| AD-1701014.1 | csasucaaAfuUfUfCfaggaccugcuL96 | 1951 | asGfscadGg(Tgn)ccugaaAfuUfugaugscsu | 2399 | AGCAUCAAAUUUCAGGACCUGCA | 2847 |
| AD-1701016.1 | asusuucaGfgAfCfCfugcagacaguL96 | 1952 | asCfsugdTc(Tgn)gcagguCfcUfgaaaususu | 2400 | AAAUUUCAGGACCUGCAGACAGU | 2848 |
| AD-1701017.1 | ususucagGfaCfCfUfgcagacaguuL96 | 1953 | asAfscudGu(Cgn)ugcaggUfcCfugaaasusu | 2401 | AAUUUCAGGACCUGCAGACAGUA | 2849 |
| AD-1701019.1 | csasggacCfuGfCfAfgacaguacauL96 | 1954 | asUfsgudAc(Tgn)gucugcAfgGfuccugsasa | 2402 | UUCAGGACCUGCAGACAGUACAG | 2850 |
| AD-1701020.1 | gsasccugCfaGfAfCfaguacaggcuL96 | 1955 | asGfsccdTg(Tgn)acugucUfgCfaggucscsu | 2403 | AGGACCUGCAGACAGUACAGGCU | 2851 |
| AD-1701022.1 | cscsugcaGfaCfAfGfuacaggcuauL96 | 1956 | asUfsagdCc(Tgn)guacugUfcUfgcaggsusc | 2404 | GACCUGCAGACAGUACAGGCUAG | 2852 |
| AD-1701023.1 | csusgcagAfcAfGfUfacaggcuaguL96 | 1957 | asCfsuadGc(Cgn)uguacuGfuCfugcagsgsu | 2405 | ACCUGCAGACAGUACAGGCUAGA | 2853 |
| AD-1701024.1 | usgscagaCfaGfUfAfcaggcuagauL96 | 1958 | asUfscudAg(Cgn)cuguacUfgUfcugcasgsg | 2406 | CCUGCAGACAGUACAGGCUAGAU | 2854 |
| AD-1701026.1 | csasgacaGfuAfCfAfggcuagauauL96 | 1959 | asUfsaudCu(Agn)gccuguAfcUfgucugscsa | 2407 | UGCAGACAGUACAGGCUAGAUAA | 2855 |
| AD-1701027.1 | gsascaguAfcAfGfGfcuagauaacuL96 | 1960 | asGfsuudAu(Cgn)uagccuGfuAfcugucsusg | 2408 | CAGACAGUACAGGCUAGAUAACC | 2856 |
| AD-1701029.1 | cscsuagcCfaCfCfUfuucuggccuuL96 | 1961 | asAfsggdCc(Agn)gaaaggUfgGfcuaggsasa | 2409 | UUCCUAGCCACCUUUCUGGCCUC | 2857 |
| AD-1701031.1 | csusuucuGfgCfCfUfcccacuugcuL96 | 1962 | asGfscadAg(Tgn)gggaggCfcAfgaaagsgsu | 2410 | ACCUUUCUGGCCUCCCACUUGCC | 2858 |
| AD-1701032.1 | uscsuggcCfuCfCfCfcfacuugcccuuL96 | 1963 | asAfsggdGc(Agn)agugggAfgGfccagasasa | 2411 | UUUCUGGCCUCCCACUUGCCCUG | 2859 |
| AD-1701033.1 | gsgscaucUfuGfAfUfcccagccuguL96 | 1964 | asCfsagdGc(Tgn)gggaucAfaGfaugccsusg | 2412 | CAGGCAUCUUGAUCCCAGCCUGA | 2860 |
| AD-1701034.1 | gscsaucuUfgAfUfCfccagccugauL96 | 1965 | asUfscadGg(Cgn)ugggauCfaAfgaugcscsu | 2413 | AGGCAUCUUGAUCCCAGCCUGAC | 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 | ascscaauGfcCfUfCfcuguccccuauL96 | 1966 | asUfsagdGg(Agn)caggagGfcAfuuggusasg | 2414 | CUACCAAUGCCUCCUGUCCCUAA | 2862 |
| AD-1701037.1 | asusgccuCfcUfGfUfcccuaaacuuL96 | 1967 | asAfsgudTu(Agn)gggacaGfgAfggcaususg | 2415 | CAAUGCCUCCUGUCCCUAAACUC | 2863 |
| AD-1701038.1 | gscscuccUfgUfCfCfcuaaacuccuL96 | 1968 | asGfsgadGu(Tgn)uagggaCfaGfgaggcsasu | 2416 | AUGCCUCCUGUCCCUAAACUCCC | 2864 |
| AD-1720280.1 | asasaacccuCfGfAfgaccucuuuuL96 | 1969 | asdAsadAg(Agn)ggucudCgAfgGfguuuusgsc | 2417 | GCAAAACCCUCGAGACCUCUUUG | 2865 |
| AD-1720281.1 | csusgggcugAfGfAfucccaggguuuL96 | 1970 | asdAsadCc(Tgn)gggaudCuCfaGfcccagsgsa | 2418 | AGGAAAGAGCAGAUCCCAGGUUU | 2866 |
| AD-1720282.1 | gsusggacagUfGfAfggacuucuuuL96 | 1971 | asdAsadGa(Agn)guccudCaCfuGfuccacsusg | 2419 | CAGUGGACAGUGAGGACUUCUUC | 2867 |
| AD-1720283.1 | csasaaacccUfCfGfagaccucuuuL96 | 1972 | asdAsagadGg(Tgn)cucdGaGfgGfuuuugscsu | 2420 | AGCAAAACCCUCGAGACCUCUUU | 2868 |
| AD-1720284.1 | csusgaauguCfAfAfagccacauuuL96 | 1973 | asdAsadTg(Tgn)ggcuudTgAfcAfuucagsgsc | 2421 | GCCUGAAUGUCAAAGCCACAUUC | 2869 |
| AD-1720285.1 | gsgscucuAfcUfCfUfaugaguuguuL96 | 1974 | asdAscadAc(Tgn)cauadGaGfuAfgagccscsg | 2422 | CGGGCUCUACUCUAUGAGUUGUG | 2870 |
| AD-1720286.1 | asasuggaguGfCfUfaacccugguuL96 | 1975 | asdAscc(Agn)gdGguuadGcAfcUfccauuscsa | 2423 | UGAAUGGAGUGCUAACCCUGGUG | 2871 |
| AD-1720287.1 | asasuggaguGfCfUfaaccuugguuL96 | 1976 | asdAsccdAadGguuadGcAfcuccauuscsa | 2424 | UGAAUGGAGUGCUAACCCUGGUG | 2872 |
| AD-1720288.1 | asasuggaguGfCfUfaacucugguuL96 | 1977 | asdAsccdAgdAguuadGcAfcuccauuscsa | 2425 | UGAAUGGAGUGCUAACCCUGGUG | 2873 |
| AD-1720289.1 | ascsccuaCfuGfCfUfgaauggaguuL96 | 1978 | asdAscudCc(Agn)uucadGcAfgUfaggguscsu | 2426 | AGACCCUACUGCUGAAUGGAGUG | 2874 |
| AD-1720290.1 | ascscugcagAfCfAfguacaggcuuL96 | 1979 | asdAsgdCc(Tgn)guacudGuCfuGfcaggcscsc | 2427 | GGACCUGCAGACAGUACAGGCUA | 2875 |
| AD-1720291.1 | csusgggaauUfUfCfcuccacccuuL96 | 1980 | asdAsgdGg(Tgn)ggaggdAaAfuUfcccagscsa | 2428 | UGCUGGGAAUUUCCUCCACCCUU | 2876 |
| AD-1720292.1 | gsgsgccgccUfCfCfauuccuacuuL96 | 1981 | asdAsguadGg(Agn)augdGaGfgCfggcccsusu | 2429 | AAGGGCCGCCUCCAUUCCUACUA | 2877 |
| AD-1720293.1 | ususcuacggGfCfUfcuacucuauuL96 | 1982 | asdAsudAg(Agn)guagadGcCfcGfuagaasusg | 2430 | CAUUCUACGGGCUCUACUCUAUG | 2878 |
| AD-1720294.1 | gscsagacagUfAfCfaggcuagauuL96 | 1983 | asdAsudCu(Agn)gccugdTaCfuGfucugcsasg | 2431 | CUGCAGACAGUACAGGCUAGAUA | 2879 |
| AD-1720295.1 | usgsgagaccCfUfAfcugcugaauuL96 | 1984 | asdAsudTc(Agn)gcagudAgGfgUfcuccasasu | 2432 | AUUGGAGACCCUACUGCUGAAUG | 2880 |
| AD-1720296.1 | ascscucuuuGfGfCfagccugaauuL96 | 1985 | asdAsudTc(Agn)ggcugdCcAfaAfgaggcscsu | 2433 | AGACCUCUUUGGCAGCCUGAAUG | 2881 |
| AD-1720297.1 | cscsuuucugGfCfCfucccacuuguL96 | 1986 | asdCsadAg(Tgn)gggagdGcCfaGfaaaggsusg | 2434 | CACCUUUCUGGCCUCCCACUUGC | 2882 |
| AD-1720298.1 | usgsgaacUfgCfAfGfuggacaguguL96 | 1987 | asdCsacdTg(Tgn)ccacdTgCfaGfuuccasusc | 2435 | GAUGGAACUGCAGUGGACAGUGA | 2883 |
| AD-1720299.1 | asgsgaugacAfCfGfugccugauguL96 | 1988 | asdCsadTc(Agn)ggcacdGuGfuCfauccuscsc | 2436 | GGAGGAUGACACGUGCCUGAUGG | 2884 |
| AD-1720300.1 | gsasauggagUfGfCfuaacccugguL96 | 1989 | asdCscagdGg(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 | gsusgcugucAfUfAfuggccuggguL96 | 1990 | asdCscc(Agn)gdGccaudAuGfaCfagcacsusc | 2438 | GAGUGCUGUCAUAUGGCCUGGGA | 2886 |
| AD-1720302.1 | gsusgcugucAfUfAfuggcuuggguL96 | 1991 | asdCsccdAadGccaudAuGfacagcacsusc | 2439 | GAGUGCUGUCAUAUGGCCUGGGA | 2887 |
| AD-1720303.1 | gsusgcugucAfUfAfuggucuggguL96 | 1992 | asdCsccdAgdAccaudAuGfacagcacsusc | 2440 | GAGUGCUGUCAUAUGGCCUGGGA | 2888 |
| AD-1720304.1 | gscsccaaagAfAfAfguacucagguL96 | 1993 | asdCscdTg(Agn)guacudTuCfuUfugggcscsa | 2441 | UGGCCCAAAGAAAGUACUCAGGG | 2889 |
| AD-1720305.1 | gsusacucagGfGfAfgcuccuucguL96 | 1994 | asdCsgaadGg(Agn)gcudCcCfuGfaguacsusu | 2442 | AAGUACUCAGGGAGCUCCUUCGU | 2890 |
| AD-1720306.1 | gsgsaauuucCfUfCfcacccuucguL96 | 1995 | asdCsga(Agn)gdGguggdAgGfaAfauuccscsa | 2443 | UGGGAAUUUCCUCCACCCUUCGU | 2891 |
| AD-1720307.1 | gsgsaauuucCfUfCfcaccuuucguL96 | 1996 | asdCsgadAadGguggdAgGfaaauuccscsa | 2444 | UGGGAAUUUCCUCCACCCUUCGU | 2892 |
| AD-1720308.1 | gsgsaauuucCfUfCfcacucuucguL96 | 1997 | asdCsgadAgdAguggdAgGfaaauuccscsa | 2445 | UGGGAAUUUCCUCCACCCUUCGU | 2893 |
| AD-1720309.1 | gsusguacaaGfCfAfaaacccucguL96 | 1998 | asdCsgagdGg(Tgn)uuudGcUfuGfuacacsgsu | 2446 | ACGUGUACAAGCAAAACCCUCGA | 2894 |
| AD-1720310.1 | csasgucuggUfCfAfgagcuggaguL96 | 1999 | asdCsudCc(Agn)gcucudGaCfcAfgacugscsa | 2447 | UGCAGUCUGGUCAGAGCUGGAGC | 2895 |
| AD-1720311.1 | usgscuaaccCfUfGfgugcuagaguL96 | 2000 | asdCsudCu(Agn)gcaccdAgGfgUfuagcascsu | 2448 | AGUGCUAACCCUGGUGCUAGAGG | 2896 |
| AD-1720312.1 | uscsaaauuuCfAfGfgaccugcaguL96 | 2001 | asdCsudGc(Agn)gguccdTgAfaAfuuugasusg | 2449 | CAUCAAAUUUCAGGACCUGCAGA | 2897 |
| AD-1720313.1 | asgsaaccacCfCfAfgcccagaaguL96 | 2002 | asdCsudTc(Tgn)gggcudGgGfuGfguucuscsu | 2450 | AGAGAACCACCCAGCCCAGAAGU | 2898 |
| AD-1720314.1 | csasaacuaaCfAfAfaaacauuucuL96 | 2003 | asdGsadAa(Tgn)guuuudTgUfuAfguuugsasg | 2451 | CUCAAACUAACAAAAACAUUUCC | 2899 |
| AD-1720315.1 | csasuggaguAfCfCfucucagcucuL96 | 2004 | asdGsadGc(Tgn)gagagdGuAfcUfccaugsgsu | 2452 | ACCAUGGAGUACCUCUCAGCUCU | 2900 |
| AD-1720316.1 | uscsguuggaCfCfUfccacacugcuL96 | 2005 | asdGscdAg(Tgn)guggadGgUfcCfaacgasasg | 2453 | CUUCGUUGGACCUCCACACUGCU | 2901 |
| AD-1720317.1 | ascscauggaGfUfAfccucucagcuL96 | 2006 | asdGscdTg(Agn)gaggudAcUfcCfauggusgsg | 2454 | CCACCAUGGAGUACCUCUCAGCU | 2902 |
| AD-1720368.1 | gsgscagccuGfAfAfugucaaagcuL96 | 2007 | asdGscdTu(Tgn)gacaudTcAfgGfcugccsasa | 2455 | UUGGCAGCCUGAAUGUCAAAGCC | 2903 |
| AD-1720369.1 | uscscuagccAfCfCfuuucuggccuL96 | 2008 | asdGsgdCc(Agn)gaaagdGuGfgCfuaggasasa | 2456 | UUUCCUAGCCACCUUUCUGGCCU | 2904 |
| AD-1720370.1 | usgscugaauGfGfAfgugcuaaccuL96 | 2009 | asdGsgdTu(Agn)gcacudCcAfuUfcagcasgsu | 2457 | ACUGCUGAAUGGAGUGCUAACCC | 2905 |
| AD-1720371.1 | uscsaggaccUfGfCfagacaguacuL96 | 2010 | asdGsudAc(Tgn)gucugdCaGfgUfccugasasa | 2458 | UUUCAGGACCUGCAGACAGUACA | 2906 |
| AD-1720372.1 | ascsagcaucAfAfAfuuucaggacuL96 | 2011 | asdGsudCc(Tgn)gaaaudTuGfaUfgcuguscsa | 2459 | UGACAGCAUCAAAUUUCAGGACC | 2907 |
| AD-1720373.1 | ususgcugggAfAfUfuuccuccacuL96 | 2012 | asdGsudGg(Agn)ggaaadTuCfcCfagcaascsa | 2460 | UGUUGCUGGGAAUUUCCUCCACC | 2908 |
| AD-1720374.1 | asasugccucCfUfGfucccuaaacuL96 | 2013 | asdGsudTu(Agn)gggacdAgGfaGfgcauusgsg | 2461 | CCAAUGCCUCCUGUCCCUAAACU | 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 | ususgacgugUfAfCfaagcaaaacuL96 | 2014 | asdGsudTu(Tgn)gcuugdTaCfaCfgucaasasg | 2462 | CUUUGACGUGUACAAGCAAAACC | 2910 |
| AD-1720376.1 | gsgsccgccuCfCfAfuuccuacuauL96 | 2015 | asdTsadGu(Agn)ggaaudGgAfgGfcggccscsu | 2463 | AGGGCCGCCUCCAUUCCUACUAA | 2911 |
| AD-1720377.1 | ascsucagguCfAfGfuaucuaauauL96 | 2016 | asdTsadTu(Agn)gauacdTgAfcCfugagusasa | 2464 | UUACUCAGGUCAGUAUCUAAUAU | 2912 |
| AD-1720378.1 | usgsggccauAfUfGfuugcugggauL96 | 2017 | asdTscdCc(Agn)gcaacdAuAfuGfgcccasgsg | 2465 | CCUGGGCCAUAUGUUGCUGGGAA | 2913 |
| AD-1720379.1 | usgscugucaUfAfUfggccugggauL96 | 2018 | asdTscdCc(Agn)ggccadTaUfgAfcagcascsu | 2466 | AGUGCUGUCAUAUGGCCUGGGAC | 2914 |
| AD-1720380.1 | usgsaguuguGfAfCfuuucaaggauL96 | 2019 | asdTscdCu(Tgn)gaaagdTcAfcAfacucasusa | 2467 | UAUGAGUUGUGACUUUCAAGGAC | 2915 |
| AD-1720381.1 | asgsgcccaaGfCfAfcagcaaggauL96 | 2020 | asdTscdCu(Tgn)gcugudGcUfuGfggccuscsu | 2468 | AGAGGCCCAAGCACAGCAAGGAC | 2916 |
| AD-1720382.1 | usgsuacaagCfAfAfaacccucgauL96 | 2021 | asdTscg(Agn)gdGguuudTgCfuUfguacascsg | 2469 | CGUGUACAAGCAAAACCCUCGAG | 2917 |
| AD-1720383.1 | usgsuacaagCfAfAfaaccuucgauL96 | 2022 | asdTscgdAadGguuudTgCfuuguacascsg | 2470 | CGUGUACAAGCAAAACCCUCGAG | 2918 |
| AD-1720384.1 | usgsuacaagCfAfAfaacucucgauL96 | 2023 | asdTscgdAgdAguuudTgCfuuguacascsg | 2471 | CGUGUACAAGCAAAACCCUCGAG | 2919 |
| AD-1720385.1 | uscsuaugagUfUfGfugacuuucauL96 | 2024 | asdTsgdAa(Agn)gucacdAaCfuCfauagasgsu | 2472 | ACUCUAUGAGUUGUGACUUUCAA | 2920 |
| AD-1720386.1 | asasagacuaUfGfAfcagcaucaauL96 | 2025 | asdTsudGa(Tgn)gcugudCaUfaGfucuuusgsc | 2473 | GCAAAGACUAUGACAGCAUCAAA | 2921 |

**Example 2. *In vitro* screening of CIDEB siRNA**

**Experimental Methods**

**Cell culture and transfections:**

Hepa1c1c7 cells are grown to near confluence at 37°C in an atmosphere of 5% $CO_2$ 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 complete growth media without antibiotic containing ~2 x$10^4$ 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% $CO_2$ 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 14.6 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 µl of complete growth media without antibiotic containing ~1.5 x$10^4$ 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% $CO_2$ 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 µl of Opti-MEM plus 0.4 µl of Lipofectamine RNAimax per well to 5 µl 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 µl of complete growth media without antibiotic containing ~1.5 x$10^4$ 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 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 150 µl Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150 µl Elution Buffer, re-captured and supernatant removed.

**Synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813):**

Ten µl of a master mix containing 1 µl 10X Buffer, 0.4 µl 25X dNTPs, 1 µl 10x Random primers, 0.5 µl Reverse Transcriptase, 0.5 µl RNase inhibitor and 6.6 µl of $H_2O$ 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 µl of cDNA and 5 µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) were added to either 0.5 µl of human GAPDH TaqMan Probe and 0.5 µl human CIDEB 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 ΔΔCt 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.

**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 |

| | 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-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 |

| | 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-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 |

| | 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-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 |

| | 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-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 |

| | 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-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 |

| | 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-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 cynomologus 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 0.1µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl of siRNA duplexes per well into a 384-well plate and incubated at room temperature for 15 minutes. 40µl of in Invitrogro CP media (BioIVT, Cat # Z99029) containing ~10 x$10^3$ 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 µL 10X Buffer, 0.4 µL 25X deoxyribonucleotide triphosphate, 1 µL 10X Random primers, 0.5 µL Reverse Transcriptase, 0.5 µL RNase inhibitor, and 6.6 µL of $H_2O$ 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. 2µl of cDNA were added to a master mix containing 0.5µl of human or cyno GAPDH TaqMan Probe, 0.5µl human or cyno CIDEB probe) and 5µl 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 of the single dose transfection screens and free uptake screens of the dsRNA agents in PCH is showin 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 x $10^{10}$ 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 (±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 | | |



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| 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 x $10^{10}$ 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 (±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 | | |



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| 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

> NM_001393338.1 Homo sapiens cell death inducing DFFA like effector b (CIDEB), transcript variant 7, mRNA

CCCTTCCGGTGGAGCCAGCGCTGCGACCGCCTGCAGAAGGTTGACTGCGTGGTAGGGGGCCCAGAGCAAGCCGAAGG
CAAGCACGATGGCGCTCACCAGCCGGCCCACCCGCGCCCCGTGCCGCCCGGAGCCCCAGCGGGCGCCCCGCAGCCGT
GCCAGCGTCACGCTGTAGCAGCCGAGCATCAGCCCGAAAGGAAGCACGAAAGCGGTCAGAGTCTCCAGGCTCAGGTG
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CCGCCAGGAGCTGCTAGCCAAAGCATTGGAGACCCTACTGCTGAATGGAGTGCTAACCCTGGTGCTAGAGGAGGATG
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CCGATTCACCTTTGACGTGTACAAGCAAAACCCTCGAGACCTCTTTGGCAGCCTGAATGTCAAAGCCACATTCTACG
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SEQ ID NO:2

>Reverse complement of SEQ ID NO: 1

TTAGTGGTAAATATTTGATATTTTTATTGGAAATGTTTTTGTTAGTTTGAGGGGAAGGGTATGAAGACAGATCTCAA
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CAGCAGTCAGGCTGGGATCAAGATGCCTGGGGGACATCTTGATCTTGGCCTTTCAGGGCAAGTGGGAGGCCAGAAAG
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CCGGTCCACGCCGCCGCCCACCTGAGCCTGGAGACTCTGACCGCTTTCGTGCTTCCTTTCGGGCTGATGCTCGGCTG
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TGAGCGCCATCGTGCTTGCCTTCGGCTTGCTCTGGGCCCCCTACCACGCAGTCAACCTTCTGCAGGCGGTCGCAGCG
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.

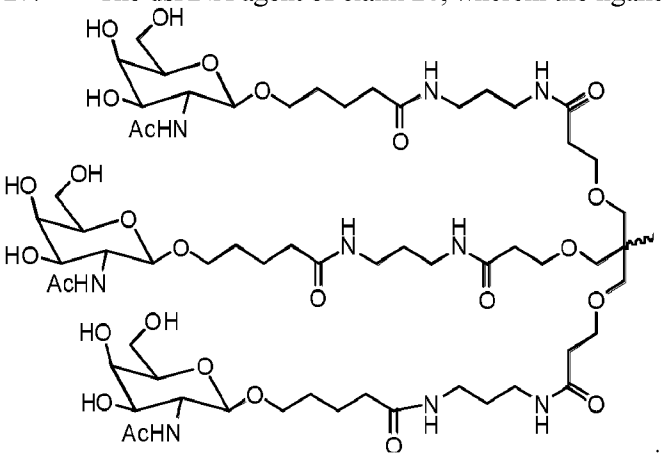
23. The dsRNA agent of any one of claim 22, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

24. The dsRNA agent of any one of claims 1-7 and 12-23 further comprising a ligand.

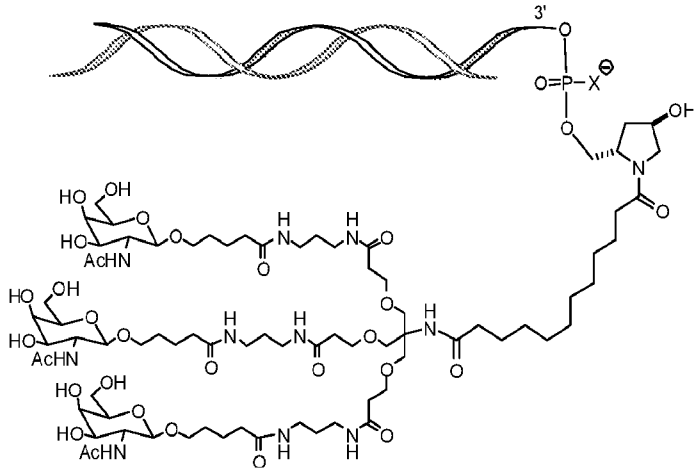
25. The dsRNA agent of claim 24, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

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):

sense: $5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (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'-flouro 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.

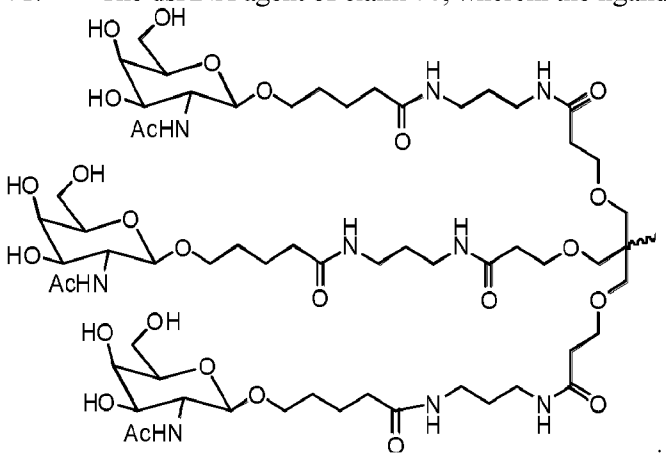
67. The dsRNA agent of claim 66, wherein all $n_p'$ are linked to neighboring nucleotides via phosphorothioate linkages.

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.

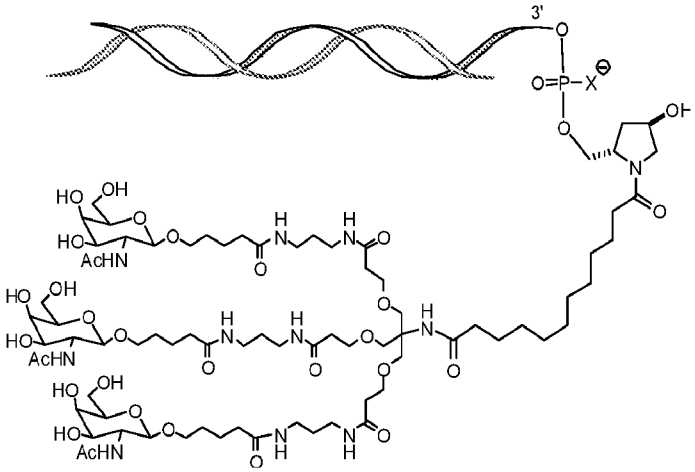
69. The dsRNA agent of any one of claims 31-68, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

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):

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;

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):

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 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):

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 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):

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 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 (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 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 CIDEB in the subject.