(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 January 2017 (19.01.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/011286 A1

(51) International Patent Classification:
C12N 15/113 (2010.01) *A61K 31/712* (2006.01)

(21) International Application Number:
PCT/US2016/041440

(22) International Filing Date:
8 July 2016 (08.07.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/191,008	10 July 2015 (10.07.2015)	US
62/269,401	18 December 2015 (18.12.2015)	US
62/316,726	1 April 2016 (01.04.2016)	US

(71) Applicant: **ALNYLAM PHARMACEUTICALS, INC.** [US/US]; 300 Third Street, 3rd Floor, Cambridge, MA 02142 (US).

(72) Inventors: **BUTLER, James**; 300 Third Street, 3rd Floor, Cambridge, MA 02142 (US). **FITZGERALD, Kevin**; 300 Third Street, 3rd Floor, Cambridge, MA 02142 (US). **HINKLE, Gregory**; 300 Third Street, 3rd Floor, Cambridge, MA 02142 (US). **BETTENCOURT, Brian**; 300 Third Street, 3rd Floor, Cambridge, MA 02142 (US). **XU, Huilei**; 300 Third Street, 3rd Floor, Cambridge, MA 02142 (US).

(74) Agents: **ZACHARAKIS, Maria, Laccotripe** et al.; McCarter & English, LLP, 265 Franklin Street, Boston, MA 02110 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2017/011286 A1

(54) **Title**: INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN, ACID LABILE SUBUNIT (IGFALS) AND INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) IRNA COMPOSITIONS AND METHODS OF USE THEREOF

(57) **Abstract**: The present invention relates to RNAi agents, e.g., double stranded RNAi agents, targeting the insulin-like growth factor binding protein, acid labile subunit (IGFALS) gene or the insulin-like growth factor 1 (IGF-1) gene, methods of using such double stranded RNAi agents to inhibit expression of an IGFALS gene or an IGF-1 gene, and methods of treating subjects having an IGF system-associated disorder.

INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN, ACID LABILE SUBUNIT (IGFALS) AND INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) iRNA COMPOSITIONS AND METHODS OF USE THEREOF

Related Application

This application is related to U.S. Provisional Patent Application Nos.: 62/191,008, filed on July 10, 2015; 62/269,401, filed on December 18, 2015; and 62/316,726, filed on April 1, 2016. The entire contents of each 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 ASCII format and is hereby incoroporated by reference in its entirety. The ASCII copy, created on July 7, 2016, is named 121301-03920_SL.txt and is 1,164,878 bytes in size.

Background of the Invention

Acromegaly is a progressive and life threatening disease resulting from growth hormone hypersecretion from a benign pituitary tumor, leading to approximately a 10 year reduction in lifespan and a reduced quality of life. Acromegaly is associated with cardiovascular disease including hypertension and cardiac hypertrophy, cerebrovascular disease including stroke, metabolic disease including diabetes, and respiratory disease including sleep apnea. Mortality rates in acromegaly are correlated with growth hormone and IGF-1 levels, with increased growth hormone concentrations being associated with shorter life spans (Holdaway *et al.*, JCEM, 2004). The clinical features most commonly associated with acromegaly are acral enlargement, maxofacial changes, excessive sweating, athralgias, headache, hypogonadal symptoms, visual deficit, fatigue, weight gain, and galactorrhea. Such symptoms may be associated with any of a number of diseases or conditions and, thus, diagnosis of acromegaly often does not occur until several years after the initiation of growth hormone hypersecretion. Definitive diagnosis of acromeagly includes detection of an increased level of insulin-like growth factor-1 (IGF-1) and growth hormone elevation in an oral glucose tolerance test, confirmed by detection of a GH-hypersecreting pituitary tumor, typically by MRI. (The diagnostic criteria for acromegaly are provided in the American Association of Clinical Endocrinologists Medical Guidelines for Clinical Practice for the Diagnosis and Treatment of Acromegaly – 2011 Update (Katznelson *et al.*, *Endocr. Pract.* 17 (Suppl. 4)).

Current treatment options for acromegaly are insufficient for many patients. Surgical removal of the pituitary adenoma by transsphenoidal surgery results in a cure for about 50-60% of patients. Subjects for whom surgical intervention is not possible or does not result in a cure are treated with first-line pharmacological therapy which includes dopamine agonists or sustained-release somatostatin analogs (SSAs). This therapy results in good control for the disease for about 70% of these patients for whom surgery cannot provide a cure. The use of SSAs, however, is limited

to subjects expressing a somatostatin receptor on their tumor. Subjects whose disease cannot be controlled by the first-line pharmacological therapy are treated with SOMAVERT® (pegvisomant), a growth hormone receptor antagonist, which is administered by daily subcutaneous injection. Radiotherapy, which suffers from low efficacy and high side effects, is used as a last resort.

The insulin-like growth factor system is also associated with abnormal growth in cancer and metastasis (see, *e.g.*, Samani *et al.*, Endocrine Rev., 2007). The IGF system has become a target for anticancer agents, both as primary and adjunctive therapy.

Currently, treatments for acromegaly and cancer do not fully meet patient needs. Therefore, there is a need for therapies for subjects suffering from acromegaly or cancer.

Summary of the Invention

The present invention provides iRNA compositions which affect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an insulin-like growth factor binding protein, acid labile subunit (IGFALS) gene or an insulin-like growth factor-1(IGF-1) gene. The IGFALS gene or IGF-1 gene may be within a cell, *e.g.*, a cell within a subject, such as a human.

In an aspect, the invention provides a double stranded ribonucleic acid interference (dsRNA) agent for inhibiting expression of insulin-like growth factor binding protein, acid labile subunit (IGFALS), wherein the double stranded 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.

In certain embodiments, the sense strands and antisense strands comprise sequences selected from any one of the sequences in any one of Tables 3, 5, 6, 8, 12, or 14.

In an aspect, the invention provides a double stranded ribonucleic acid interference (dsRNAi) agent for inhibiting expression of insulin-like growth factor-1 (IGF-1), wherein the double stranded RNAi 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: 11 or 13 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: 12 or 14.

In certain embodiments, the sense strands and antisense strands comprise sequences selected from any one of the sequences in any one of Tables 9, 11, 15, 17, 18, or 20.

In an aspect, the invention provides a double stranded ribonucleic acid interference (dsRNAi) agent for inhibiting expression of insulin-like growth factor binding protein, acid labile subunit (IGFALS), wherein the double stranded RNAi 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 3 nucleotides from any one of the antisense sequences listed in any one of Tables 3, 5, 6, 8, 12, or 14.

In an aspect, the invention provides a double stranded ribonucleic acid interference (dsRNAi) agent for inhibiting expression of insulin-like growth factor 1 (IGF-1) wherein the double stranded RNAi 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 3 nucleotides from any one of the antisense sequences listed in any one of Tables 9, 11, 15, 17, 18, or 20.

In certain embodiments, the double stranded RNAi comprises at least one modified nucleotide. In some embodiments, substantially all of the nucleotides of the sense strand are modified nucleotides. In some embodiments, substantially all of the nucleotides of the antisense strand are modified nucleotides. In some embodiments, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In an aspect, the invention provides a double stranded RNAi agent for inhibiting expression of insulin-like growth factor binding protein, acid labile subunit (IGFALS), wherein the double stranded RNAi 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 double stranded RNAi agent comprises a ligand, *e.g.,* the sense strand of the double stranded RNAi agent is conjugated to a ligand, *e.g.,* a ligand is attached at the 3'-terminus of the sense strand.

In an aspect, the invention provides a double stranded ribonucleic acid (RNAi) agent for inhibiting expression of insulin-like growth factor 1 (IGF-1), wherein the double stranded RNAi 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: 11 or 13 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: 12 or 14, 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.

Accordingly, in certain embodiments, the present invention provides double stranded RNAi agents for inhibiting expression of IGFALS, which comprise 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 nucleotides 11-62, 24-62, 79-117, 79-130, 155-173, 194-216, 194-229, 211-229, 232-293, 254-272, 310-328, 310-349, 324-345, 331-349, 353-371, 353-394, 376-394, 407-425, 439-449, 431-470, 484-515, 497-515, 541-580, 547-568, 596-647, 616-634, 673-691, 694-712, 694-734, 777-799, 781-799, 825-843, 825-855, 869-922, 958-976, 958-988, 1064-1085, 1064-1096, 1067-1085, 1067-1096, 1100-1141, 1111-1129, 1145-1163, 1145-1186,

1159-1186, 1168-1196, 1168-1214,1193-1214, 1266-1307, 1321-1339, 1342-1373, 1375-1406, 1432-1450, 1454-1472, 1519-1537, 1519-1559, 1534-1555, 1541-1559, 1606-1624, 1606-1637, 1613-1635, 1672-1690, 1672-1712, 1749-1779, 1783-1801, 1805-1823, 1806-1829, 1871-1889, 1871-1919, 1949-1977, 1993-2011, 2013-2042, 2048-2077, 2048-2088, or 2052-2084 of SEQ ID NO: 1, and, in certain embodiments, the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the corresponding position of the nucleotide sequence of SEQ ID NO: 2 such that the antisense strand is complementary to the at least 15 contiguous nucleotides differing by no more than 3 nucleotides in the sense strand..

Accordingly, in certain embodiments, the present invention provides double stranded RNAi agents for inhibiting expression of insulin-like growth factor 1 (IGF-1), which comprise 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 nucleotides 330-369, 342-369, 432-490, 432-482, 436-462, 534-559, 330-350, 342-362, 348-368, 349-369, 432-452, 435-455, 436-456, 438-458, 440-460, 441-461, 442-462, 449-469, 455-475, 460-480, 461-481, 462-482, 464-484, 470-490, 484-501, 534-554, 536-556, 538-558, 539-559, 542-562, 548-568, 577-597, 582-602, or 640-660 of the nucleotide sequence of SEQ ID NO: 11, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the corresponding position of the nucleotide sequence of SEQ ID NO: 12 such that the antisense strand is complementary to the at least 15 contiguous nucleotides differing by no more than 3 nucleotides in the sense strand.

In certain embodiments, the present invention provides double stranded RNAi agents for inhibiting expression of insulin-like growth factor 1 (IGF-1), which comprise 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 nucleotides 6-90, 127-145, 185-238, 247-265, 277-295, 389-417, 430-480, 543-561, 654-690, 750-768, 774-870, 894-930, 1007-1029, 1075-1126, 1144-1162, 1197-1215, 1232-1250, 1293-1311, 1334-1352, 1388-1458, 1463-1490, 1511-1529, 1599-1617, 1643-1661, 1690-1727, 1793-1825, 1843-1861, 2057-2075, 2090-2130, 2192-2228, 2310-2332, 2357-2375, 2521-2539, 2566-2588, 2648-2684, 2793-2811, 2962-2980, 3120-3142, 3208-3233, 3269-3287, 3417-3435, 3449-3467, 3575-3603, 3686-3704, 3721-3739, 3806-3824, 3939-3957, 3982-4018, 4081-4037, 4154-4172, 4271-4289, 4319-4377, 4436-4478, 4484-4502, 4523-4545, 4566-4584, 4610-4660, 4686-4717, 4734-4769, 4780-4798, 4815-4843, 4884-4902, 4911-4929, 5004-5034, 5050-5068, 5171-5256, 5311-5364, 5409-5430, 5551-5588, 5609-5638, 5694-5712, 5715-5758, 5790-5808, 5906-5928, 5934-5952, 6323-6345, 6399-6417, 6461-6497, 6510-6535, 6584-6612, 6629-6647, 6661-6683, 6726-6789, 6796-6824, 6826-6851, 6858-6905, 6910-6927, 7004-7022, 7035-7130, 7144-7162, 7175-7241, and 7252-7270 of the nucleotide sequence of SEQ ID NO: 13, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the corresponding position of the nucleotide sequence of SEQ ID NO: 14 such that the antisense strand is complementary to the at least 15 contiguous nucleotides differing by no more than 3 nucleotides in the sense strand.

In certain embodiments, the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 342-369, 432-462, 330-350, 342-362, 348-368, 349-369, 432-452, 435-455, 436-456, 438-458, 440-460, 442-462, 470-490, 481-501, 536-556, or 539-559 of the nucleotide sequence of SEQ ID NO: 11 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the corresponding position of the nucleotide sequence of SEQ ID NO: 12 such that the antisense strand is complementary to the at least 15 contiguous nucleotides differing by no more than 3 nucleotides in the sense strand. In certain embodiments, the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 340-369, 430-490, 430-482, 434-460, 532-559, 328-350, 340-362, 346-368, 347-369, 430-452, 433-455, 434-456, 436-458, 438-460, 439-461, 440-462, 447-469, 453-475, 458-480, 459-481, 460-482, 461-483, 462-484, 468-490, 479-501, 532-554, 534-556, 536-558, 537-559, 540-562, 546-568, 575-597, 580-602, or 638-660 of the nucleotide sequence of SEQ ID NO: 11, for example nucleotides 342-369, 432-462, 330-350, 342-362, 348-368, 349-369, 432-452, 435-455, 436-456, 438-458, 440-460, 442-462, 470-490, 481-501, 536-556, or 539-559 of the nucleotide sequence of SEQ ID NO: 11, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the corresponding position of the nucleotide sequence of SEQ ID NO: 12 such that the antisense strand is complementary to the at least 15 contiguous nucleotides differing by no more than 3 nucleotides in the sense strand.

In certain embodiments, the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 6-90, 127-145, 185-238, 247-265, 277-295, 389-417, 430-480, 543-561, 654-690, 750-768, 774-870, 894-930, 1007-1029, 1075-1126, 1144-1162, 1197-1215, 1232-1250, 1293-1311, 1334-1352, 1388-1458, 1463-1490, 1511-1529, 1599-1617, 1643-1661, 1690-1727, 1793-1825, 1843-1861, 2057-2075, 2090-2130, 2192-2228, 2310-2332, 2357-2375, 2521-2539, 2566-2588, 2648-2684, 2793-2811, 2962-2980, 3120-3142, 3208-3233, 3269-3287, 3417-3435, 3449-3467, 3575-3603, 3686-3704, 3721-3739, 3806-3824, 3939-3957, 3982-4018, 4081-4037, 4154-4172, 4271-4289, 4319-4377, 4436-4478, 4484-4502, 4523-4545, 4566-4584, 4610-4660, 4686-4717, 4734-4769, 4780-4798, 4815-4843, 4884-4902, 4911-4929, 5004-5034, 5050-5068, 5171-5256, 5311-5364, 5409-5430, 5551-5588, 5609-5638, 5694-5712, 5715-5758, 5790-5808, 5906-5928, 5934-5952, 6323-6345, 6399-6417, 6461-6497, 6510-6535, 6584-6612, 6629-6647, 6661-6683, 6726-6789, 6796-6824, 6826-6851, 6858-6905, 6910-6927, 7004-7022, 7035-7130, 7144-7162, 7175-7241, or 7252-7270 of the nucleotide sequence of SEQ ID NO: 13, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the corresponding position of the nucleotide sequence of SEQ ID NO: 14 such that the antisense strand is complementary to the at least 15 contiguous nucleotides differing by no more than 3 nucleotides in the sense strand.

In certain embodiments, substantially all of the nucleotides of the sense strand are modified. In cetain embodiments, substantially all of the nucleotides of the antisense strand are modified nucleotides. In certain embodiments, substantially all of the nucleotides of both strands are modified.

Further, in certain embodiments, the double stranded RNAi agent comprises a ligand, *e.g.,* the double stranded RNAi agent is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, a bivalent or a trivalent branched linker.

In certain embodiments, the present invention also provides double stranded RNAi agents for inhibiting expression of IGFALS, which comprise a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides from nucleotides 11-62, 24-62, 79-117, 79-130, 155-173, 194-216, 194-229, 211-229, 232-293, 254-272, 310-328, 310-349, 324-345, 331-349, 353-371, 353-394, 376-394, 407-425, 439-449, 431-470, 484-515, 497-515, 541-580, 547-568, 596-647, 616-634, 673-691, 694-712, 694-734, 777-799, 781-799, 825-843, 825-855, 869-922, 958-976, 958-988, 1064-1085, 1064-1096, 1067-1085, 1067-1096, 1100-1141, 1111-1129, 1145-1163, 1145-1186, 1159-1186, 1168-1196, 1168-1214,1193-1214, 1266-1307, 1321-1339, 1342-1373, 1375-1406, 1432-1450, 1454-1472, 1519-1537, 1519-1559, 1534-1555, 1541-1559, 1606-1624, 1606-1637, 1613-1635, 1672-1690, 1672-1712, 1749-1779, 1783-1801, 1805-1823, 1806-1829, 1871-1889, 1871-1919, 1949-1977, 1993-2011, 2013-2042, 2048-2077, 2048-2088, or 2052-2084 of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding position of the nucleotide sequence of SEQ ID NO: 2 such that the antisense strand is complementary to the at least 15 contiguous nucleotides in the sense strand.

In certain embodiments, the present invention provides double stranded ribonucleic acid (RNAi) agent for inhibiting expression of insulin-like growth factor 1 (IGF-1), which comprise a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides selected from the group consisting of nucleotides 330-369, 342-369, 432-490, 432-482, 436-462, 534-559, 330-350, 342-362, 348-368, 349-369, 432-452, 435-455, 436-456, 438-458, 440-460, 441-461, 442-462, 449-469, 455-475, 460-480, 461-481, 462-482, 464-484, 470-490, 484-501, 534-554, 536-556, 538-558, 539-559, 542-562, 548-568, 577-597, 582-602, or 640-660 of the nucleotide sequence of SEQ ID NO: 11 and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding position of the nucleotide sequence of SEQ ID NO: 12 such that the antisense strand is complementary to the at least 15 contiguous nucleotides in the sense strand.

In certain embodiments, the present invention provides double stranded RNAi agents for inhibiting expression of insulin-like growth factor 1 (IGF-1), which comprise 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 nucleotides 6-90, 127-145, 185-238, 247-265, 277-295, 389-417, 430-480, 543-561, 654-690, 750-768, 774-870, 894-930, 1007-1029, 1075-1126, 1144-1162, 1197-1215, 1232-1250, 1293-1311, 1334-1352, 1388-1458, 1463-1490, 1511-1529, 1599-1617, 1643-1661, 1690-1727, 1793-1825, 1843-1861, 2057-2075, 2090-2130, 2192-2228, 2310-2332, 2357-2375, 2521-2539, 2566-2588, 2648-2684, 2793-2811, 2962-2980, 3120-3142, 3208-3233, 3269-3287, 3417-3435, 3449-3467, 3575-3603, 3686-3704, 3721-3739, 3806-3824, 3939-

3957, 3982-4018, 4081-4037, 4154-4172, 4271-4289, 4319-4377, 4436-4478, 4484-4502, 4523-4545, 4566-4584, 4610-4660, 4686-4717, 4734-4769, 4780-4798, 4815-4843, 4884-4902, 4911-4929, 5004-5034, 5050-5068, 5171-5256, 5311-5364, 5409-5430, 5551-5588, 5609-5638, 5694-5712, 5715-5758, 5790-5808, 5906-5928, 5934-5952, 6323-6345, 6399-6417, 6461-6497, 6510-6535, 6584-6612, 6629-6647, 6661-6683, 6726-6789, 6796-6824, 6826-6851, 6858-6905, 6910-6927, 7004-7022, 7035-7130, 7144-7162, 7175-7241, and 7252-7270 of the nucleotide sequence of SEQ ID NO: 13, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the corresponding position of the nucleotide sequence of SEQ ID NO: 14 such that the antisense strand is complementary to the at least 15 contiguous nucleotides differing by no more than 3 nucleotides in the sense strand.

In certain embodimetns, the agents comprise a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides selected from the group of nucleotides 342-369, 432-462, 330-350, 342-362, 348-368, 349-369, 432-452, 435-455, 436-456, 438-458, 440-460, 442-462, 470-490, 481-501, 536-556, or 539-559 of the nucleotide sequence of SEQ ID NO:11 and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding position of the nucleotide sequence of SEQ ID NO: 12 such that the antisense strand is complementary to the at least 15 contiguous nucleotides in the sense strand.

In certan embodiments, the agents comprise a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides selected from the group of nucleotides 340-369, 430-490, 430-482, 434-460, 532-559, 328-350, 340-362, 346-368, 347-369, 430-452, 433-455, 434-456, 436-458, 438-460, 439-461, 440-462, 447-469, 453-475, 458-480, 459-481, 460-482, 461-483, 462-484, 468-490, 479-501, 532-554, 534-556, 536-558, 537-559, 540-562, 546-568, 575-597, 580-602, or 638-660 of the nucleotide sequence of SEQ ID NO: 11, for example nucleotides 342-369, 432-462, 330-350, 342-362, 348-368, 349-369, 432-452, 435-455, 436-456, 438-458, 440-460, 442-462, 470-490, 481-501, 536-556, or 539-559 of the nucleotide sequence of SEQ ID NO: 11, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding position of the nucleotide sequence of SEQ ID NO: 12 such that the antisense strand is complementary to the at least 15 contiguous nucleotides in the sense strand.

In certan embodiments, the agents comprise a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides selected from the group of nucleotides 6-90, 127-145, 185-238, 247-265, 277-295, 389-417, 430-480, 543-561, 654-690, 750-768, 774-870, 894-930, 1007-1029, 1075-1126, 1144-1162, 1197-1215, 1232-1250, 1293-1311, 1334-1352, 1388-1458, 1463-1490, 1511-1529, 1599-1617, 1643-1661, 1690-1727, 1793-1825, 1843-1861, 2057-2075, 2090-2130, 2192-2228, 2310-2332, 2357-2375, 2521-2539, 2566-2588, 2648-2684, 2793-2811, 2962-2980, 3120-3142, 3208-3233, 3269-3287, 3417-3435, 3449-3467, 3575-3603, 3686-3704, 3721-3739, 3806-3824, 3939-3957, 3982-4018, 4081-4037, 4154-4172, 4271-4289, 4319-4377, 4436-4478, 4484-4502, 4523-4545, 4566-4584, 4610-4660, 4686-4717, 4734-4769, 4780-4798, 4815-4843, 4884-4902, 4911-4929, 5004-5034, 5050-5068, 5171-5256, 5311-5364, 5409-

5430, 5551-5588, 5609-5638, 5694-5712, 5715-5758, 5790-5808, 5906-5928, 5934-5952, 6323-6345, 6399-6417, 6461-6497, 6510-6535, 6584-6612, 6629-6647, 6661-6683, 6726-6789, 6796-6824, 6826-6851, 6858-6905, 6910-6927, 7004-7022, 7035-7130, 7144-7162, 7175-7241, or 7252-7270 of the nucleotide sequence of SEQ ID NO: 13, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding position of the nucleotide sequence of SEQ ID NO: 14 such that the antisense strand is complementary to the at least 15 contiguous nucleotides in the sense strand.

In certain embodiments, substantially all of the nucleotides of the sense strand are modified nucleotides. In certain embodiments, substantially all of the nucleotides of the antisense strand are modified nucleotides. In certain embodiments, substantially all of the nucleotides of both strands are modified. In preferred embodiments, the double stranded RNAi agent comprises a ligand, *e.g.,* the double stranded RNAi agent is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, a bivalent or a trivalent branched linker.

In certain embodiments, the sense strand and the antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense sequences listed in any one of Tables 3, 5, 6, 8, 12, or 14 for IGFALS or any one of Tables 9, 11, 15, 17, 18, or 20 for IGF-1.

For example, in certain embodiments, the sense strand and the antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense nucleotide sequences selected from the group of the antisense nucleotide sequence of duplexes targeted to IGF selected from the group AD-66722, AD-66748, AD-66746, AD-66747, AD-66733, AD-66752, AD-66739, AD-66738, AD-66725, AD-66740, AD-66750, AD-66729, AD-66745, AD-66749, AD-66720, AD-66724, AD-66726, AD-66766, AD-66761, AD-66755, AD-66751, AD-66719, AD-66727, AD-66744, AD-66760, AD-66753, AD-66721, AD-66716, AD-66743, or AD-66728, , AD-77150, AD-77158, AD-74963, AD-77138, AD-75740, AD-74968, AD-74965, AD-75766, AD-75761, AD-75137, AD-74979, AD-74966, AD-75750, AD-77126, AD-74971, AD-74982, AD-77144, AD-77149, AD-75751, AD-75111, AD-77147, AD-74964, AD-74983, AD-75765, AD-74970, AD-75749, AD-77168, AD-77127, AD-75748, AD-75779, AD-75145, AD-74975, AD-77151, AD-75170, AD-75741, AD-75162, AD-74985, AD-75759, AD-75218, AD-74981, AD-75155, AD-74978, AD-77153, AD-75157, AD-75123, AD-75184, AD-77160, AD-75125, AD-75229, AD-77165, AD-75112, AD-75206, AD-75769, AD-75174, AD-75225, AD-75792, AD-75115, AD-74986, AD-77171, AD-75131, AD-77128, AD-75179, AD-75792, AD-77124, AD-75191, AD-75774, AD-75114, AD-74973, AD-77156, AD-75120, AD-75130, AD-74967, AD-75231, AD-74987, AD-77140, AD-74969, AD-75000, AD-75791, AD-75143, AD-77120, AD-77142, AD-75217, AD-75234, AD-75173, AD-75232, AD-75188, AD-75135, AD-75018, AD-77122, AD-75009, AD-75121, AD-75791, AD-77135, AD-75214, AD-74994, AD-75139, AD-75166, AD-75020, AD-77159, AD-75236, AD-77123, AD-77133, AD-74972, AD-75223, AD-75148, AD-75124, AD-75185, AD-75150, AD-74976, AD-74980, AD-75212, AD-75239, AD-75221, AD-75118, AD-75793, AD-75023, AD-75164, AD-74997, AD-74984, AD-75011, AD-75203, AD-77161, AD-75033, AD-75177,

AD-75795, AD-77146, AD-75793, AD-75788, AD-75079, AD-75152, AD-77121, AD-75237, AD-75014, AD-75755, AD-75028, AD-75091, AD-75110, AD-75230, AD-75029, AD-75099, AD-77130, AD-75224, AD-75142, AD-75760, AD-75795, AD-77136, AD-75032, AD-75757, AD-75017, AD-75151, AD-75122, AD-75002, AD-75021, AD-75005, AD-75088, AD-75153, AD-75208, AD-74977, AD-75069, AD-75107, AD-74990, AD-75061, AD-75083, AD-75116, AD-75169, AD-75058, AD-74991, AD-75041, AD-77131, AD-75772, AD-77169, AD-75133, AD-75222, AD-75007, AD-75101, AD-77137, AD-75090, AD-77148, AD-75008, AD-77134, AD-74999, AD-75048, AD-75095, AD-74974, AD-75788, AD-75057, AD-75113, AD-77172, AD-75016, AD-75186, AD-75205, AD-75238, or AD-75146 ; for example duplexes AD-66722, AD-66748, AD-66746, AD-66747, AD-66733, AD-66752, AD-66739, AD-66738, AD-66725, AD-66740, AD-66750, AD-66729, or AD-66745. In certain embodiments, nucleotide sequences selected from the group duplexes targeted to IGF selected from the group AD-66722, AD-66748, AD-66746, AD-66747, AD-66733, AD-66752, AD-66739, AD-66738, AD-66725, AD-66740, AD-66750, AD-66729, and AD-66745. In certain embodiments, the sense strand and the antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides of any one of the sense and antisense nucleotide sequences of the foregoing duplexes.

In certain embodiments, the sense strand and the antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense sequences of the duplexes targeted to IGFALS selected from the group AD-62728, AD-62734, AD-68111, AD-68709, AD-68712, AD-68715, AD-68716, AD-68717, AD-68719, AD-68720, AD-68722, AD-68725, AD-68726, AD-68730, AD-68731, AD-73782, AD-73773, AD-73765, AD-73946, AD-73947, AD-73858, AD-73797, AD-73808, AD-73906, AD-73912, AD-73848, AD-73836, AD-73818, AD-73786, AD-73862, AD-73795, AD-73766, AD-73930, AD-73825, AD-73924, AD-73802, AD-73767, AD-73771, AD-73777, AD-73793, AD-73898, AD-73784, AD-73882, AD-73803, AD-73772, AD-73907, AD-73948, AD-73890, AD-73883, AD-73770, AD-73867, AD-73931, AD-73932, AD-73787, AD-73791, AD-73880, AD-73914, AD-73849, AD-73863, AD-73920, AD-73944, AD-73841, AD-73785, AD-73804, AD-73823, AD-73885, AD-73788, AD-73865, AD-73941, AD-73859, AD-73913, AD-73892, AD-73837, AD-73842, AD-73840, AD-73813, AD-73796, AD-73875, AD-73900, AD-73922, AD-73861, AD-73816, AD-73764, AD-73868, AD-73812, AD-73826, AD-73938, AD-73843, AD-73817, AD-73943, AD-73827, AD-73937, AD-73877, AD-73833, AD-73807, AD-73819, AD-73886, AD-73919, AD-73800, AD-76171, AD-76173, AD-76203, AD-76210, AD-76172, AD-76175, AD-76209, AD-76174, AD-76208, AD-76186, AD-76177, AD-76199, AD-76197, or AD-76212.

In certain embodiments, substantially all of the nucleotides of the sense strand are modified nucleotides. In certain embodiments, substantially all of the nucleotides of the antisense strand are modified nucleotides. In certain embodiments, substantially all of the nucleotides of both strands are modified.

In one embodiment, at least one of the 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'-hydroxly-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, and a nucleotide comprising a 5'-phosphate mimic. In another embodiment, the modified nucleotides comprise a short sequence of 3'-terminal deoxy-thymine nucleotides (dT).

In certain embodiments, substantially all of the nucleotides of the sense strand are modified. In certain embodiments, substantially all of the nucleotides of the antisense strand are modified. In certain embodiments, substantially all of the nucleotides of both the sense strand and the antisense strand are modified.

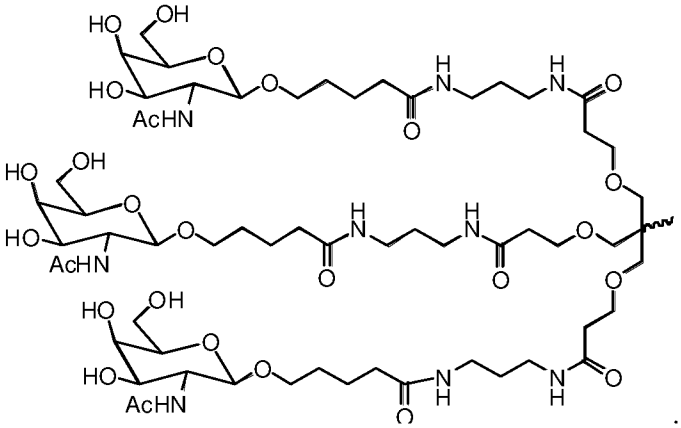
In certain embodiments, the duplex comprises a modified antisense nucleotide sequence targeted to IGFALS provided in Table 5, 8, or 14, or targeted to IGF-1 in Table 11, 17, or 20. In certain embodiments, the duplex comprises a modified sense strand nucleotide sequence targeted to IGFALS provided in Table 5, 8, or 14, or targeted to IGF-1 in Table 11, 17, or 20. In certain embodiments, the duplex comprises the modified sense strand nucleotide sequence and the modified antisense strand nucleotide of any one of the duplexes targeted to IGFALS provided in Table 5, 8, or 14, or targeted to IGF-1 in Table 11, 17, or 20.

In certain embodiments, the region of complementarity between the antisense strand and the target is at least 17 nucleotides in length. For example, the region of complementarity between the antisense strand and the target is 19 to 21 nucleotides in length, for example, the region of complementarity is 21 nucleotides in length. In preferred embodiments, each strand is no more than 30 nucleotides in length.

In some embodiments, at least one strand comprises a 3' overhang of at least 1 nucleotide, *e.g.*, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide. In other embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides.

In many embodiments, the double stranded RNAi agent further comprises a ligand. The ligand may be one or more GalNAc attached to the RNAi agent through a monovalent, a bivalent, or a

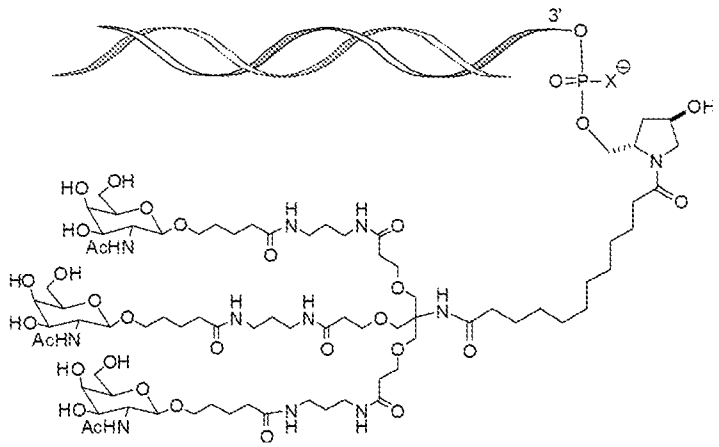
trivalent branched linker. The ligand may be conjugated to the 3' end of the sense strand of the double stranded RNAi agent. The ligand can be an N-acetylgalactosamine (GalNAc) derivative including, but not limited to



In various embodiments, the ligand is attached to the 5' end of the sense strand of the double stranded RNAi agent, the 3' end of the antisense strand of the double stranded RNAi agent, or the 5' end of the antisense strand of the double stranded RNAi agent.

In some embodiments, the double stranded RNAi agents of the invention comprise a plurality, *e.g.*, 2, 3, 4, 5, or 6, of GalNAc, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

In certain embodiments, the dsRNAi is agent 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 certain embodiments, the ligand is a cholesterol.

In certain embodiments, the region of complementarity comprises any one of the antisense sequences targeted to IGFALS provided in Table 3, 5, 6, 8, 12, or 14 or targeted to IGF-1 in Table 9, 11, 15, 17, 18, or 20. In another embodiment, the region of complementarity consists of any one of the antisense sequences of targeted to IGFALS provided in Table 3, 5, 6, 8, 12, or 14 or targeted to IGF-1 in Table 9, 11, 15, 17, 18, or 20.

In another aspect, the invention provides a double stranded RNAi agent for inhibiting expression of IGFALS or IGF-1, wherein the double stranded RNAi agent comprises a sense strand

complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding IGFALS or IGF-1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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' (III)

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 double stranded RNAi agent comprises a ligand, *e.g.,* the sense strand is conjugated to at least one ligand.

In certain embodiments, 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 another embodiment, XXX is complementary to X'X'X', YYY is complementary to Y'Y'Y', and ZZZ is complementary to Z'Z'Z'. In another embodiment, the YYY motif occurs at or near the cleavage site of the sense strand. In another embodiment, the Y'Y'Y' motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end. In one embodiment, the Y' is 2'-O-methyl.

For example, formula (III) can be represented by formula (IIIa):

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' (IIIa).

In another embodiment, formula (III) is represented by formula (IIIb):

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' (IIIb)

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

Alternatively, formula (III) can be represented by formula (IIIc):

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' (IIIc)

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

Further, formula (III) can be represented by formula (IIId):

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' (IIId)

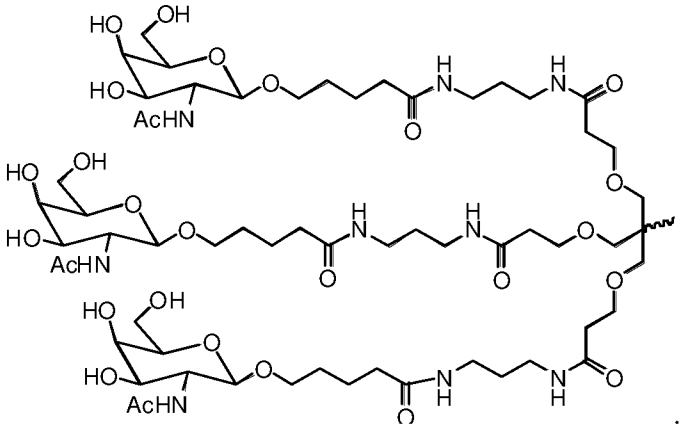
wherein each N_b and N_b' independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides and each N_a and N_a' independently represents an oligonucleotide sequence comprising 2-10 modified nucleotides.

In certain embodiment, the double stranded region is 15-30 nucleotide pairs in length. For example, the double stranded region can be 17-23 nucleotide pairs in length. The double stranded region can be 17-25 nucleotide pairs in length. The double stranded region can be 23-27 nucleotide pairs in length. The double stranded region can be 19-21 nucleotide pairs in length. The double stranded region can be 21-23 nucleotide pairs in length.

In certain embodiments, each strand has 15-30 nucleotides. In other embodiments, each strand has 19-30 nucleotides.

Modifications on the nucleotides are selected from the group including, but not limited to, LNA, HNA, CeNA, 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-deoxy, 2'-hydroxyl, and combinations thereof. In another embodiment, the modifications on the nucleotides are 2′-O-methyl or 2′-fluoro modifications.

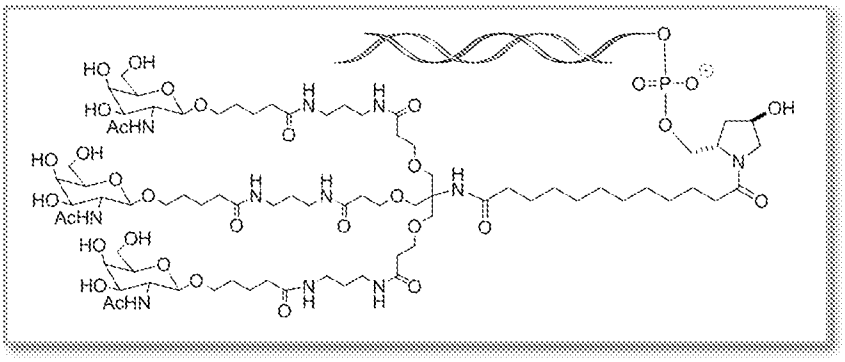
In many embodiments, the double stranded RNAi agent further comprises a ligand. The ligand may be one or more GalNAc attached to the RNAi agent through a monovalent, a bivalent, or a trivalent branched linker. The ligand may be conjugated to the 3' end of the sense strand of the double stranded RNAi agent. The ligand can be an N-acetylgalactosamine (GalNAc) derivative including, but not limited to



In various embodiments, the ligand is attached to the 5' end of the sense strand of the double stranded RNAi agent, the 3' end of the antisense strand of the double stranded RNAi agent, or the 5' end of the antisense strand of the double stranded RNAi agent.

In some embodiments, the double stranded RNAi agents of the invention comprise a plurality, *e.g.*, 2, 3, 4, 5, or 6, of GalNAc, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

An exemplary structure of a dsRNAi agent conjugated to the ligand is shown in the following schematic



In certain embodiments, the ligand can be a cholesterol.

In certain embodiments, the double stranded RNAi agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage. For example the phosphorothioate or methylphosphonate internucleotide linkage can be at the 3'-terminus of one strand, *i.e.*, the sense strand or the antisense strand; or at the ends of both strands, the sense strand and the antisense strand.

In certain embodiments, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand, *i.e.*, the sense strand or the antisense strand; or at the ends of both strands, the sense strand and the antisense strand.

In certain embodiments, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand, *i.e.*, the sense strand or the antisense strand; or at the ends of both strands, the sense strand and the antisense strand.

In certain embodiments, the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

In certain embodiments, the Y nucleotides contain a 2′-fluoro modification. In another embodiment, the Y′ nucleotides contain a 2′-O-methyl modification. In another embodiment, p′>0. In some embodiments, p′=2. In some embodiments, q'=0, p=0, q=0, and p' overhang nucleotides are complementary to the target mRNA. In some embodiments, q'=0, p=0, q=0, and p' overhang nucleotides are non-complementary to the target mRNA.

In certain embodiments, the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

In certain embodiments, at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage. In other embodiments, all n_p' are linked to neighboring nucleotides via phosphorothioate linkages.

In certain embodiments, the dsRNAi agent is selected from the group of any one of the double stranded RNAi agents targeted to IGFALS provided in Table 3, 5, 6, 8, 12, or 14, or targeted to IGF-1 in Table 9, 11, 15, 17, 18, or 20. In certain embodiments, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In an aspect, the invention provides a double stranded RNAi agent for inhibiting expression of IGFALS or IGF-1 in a cell, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region

complementary to part of an mRNA encoding IGFALS or IGF-1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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' (III)

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 double stranded RNAi agent comprises a ligand, *e.g.,* the double stranded RNAi agent is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, a bivalent or a trivalent branched linker.

In an aspect, the invention provides a double stranded RNAi agent for inhibiting expression of IGFALS or IGF-1 in a cell, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding IGFALS or IGF-1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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' (III)

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 double stranded RNAi agent comprises a ligand, *e.g.,* the double stranded RNAi

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

In certain embodiments, the invention provides a d double stranded ribonucleic acid (RNAi) agent for inhibiting expression of IGFALS or IGF-1, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding IGFALS or IGF-1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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' (III)

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 double stranded RNAi agent is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, a bivalent or a trivalent linker.

In an aspect, the invention provides a double stranded RNAi agent for inhibiting expression of IGFALS or IGF-1, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding IGFALS or IGF-1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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' (III)

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 double stranded RNAi agent comprises a ligand, *e.g.,* the double stranded RNAi agent is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, a bivalent or a trivalent branched linker.

In an aspect, the invention provides a double stranded RNAi agent capable of inhibiting the expression of IGFALS or IGF-1 in a cell, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding IGFALS of IGF-1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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′ (III)

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 double stranded RNAi agent comprises a ligand, *e.g.,* the double stranded RNAi agent is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, a bivalent or a trivalent branched linker.

In an aspect, the invention provides a double stranded RNAi agent for inhibiting expression of IGFALS or IGF-1 in a cell, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding IGFALS or IGF-1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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′ (IIIa)

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; wherein the double stranded RNAi agent comprises a ligand, *e.g.,* the double stranded RNAi agent is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, a bivalent or a trivalent branched linker.

In an aspect, the invention provides a double stranded ribonucleic acid (RNAi) agent for inhibiting expression of IGFALS, wherein the double stranded RNAi 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 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 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, a bivalent or a trivalent linker at the 3'-terminus.

In an aspect, the invention provides a double stranded ribonucleic acid (RNAi) agent for inhibiting expression of insulin-like growth factor 1 (IGF-1), wherein the double stranded RNAi 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:11 or 13 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:12 or 14, wherein substantially all of the nucleotides of the sense strand comprise a modification selected from 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 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, a bivalent or a trivalent linker at the 3'-terminus.

In certain embodiments, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides. In certain embodiments, each strand has 19-30 nucleotides.

In certain embodiments, substantially all of the nucleotides of the sense strand are modified. In certain embodiments, substantially all of the nucleotides of the antisense strand are modified. In certain embodiments, substantially all of the nucleotides of both the sense strand and the antisense strand are modified.

In an aspect, the invention provides a cell containing the dsRNAi agent as described herein.

In an aspect, the invention provides a vector encoding at least one strand of a dsRNAi agent, wherein the RNAi agent comprises a region of complementarity to at least a part of an mRNA encoding IGFALS or IGF-1, wherein the RNAi is 30 base pairs or less in length, and wherein the RNAi agent targets the mRNA for cleavage. In certain embodiments, the region of complementarity is at least 15 nucleotides in length. In certain embodiments, the region of complementarity is 19 to 23 nucleotides in length.

In an aspect, the invention provides a cell comprising a vector as described herein.

In an aspect, the invention provides a pharmaceutical composition for inhibiting expression of an IGFALS or IGF-1 gene, comprising a double stranded RNAi agent of the invention. In one embodiment, the RNAi agent is administered in an unbuffered solution. In certain embodiments, the unbuffered solution is saline or water. In other embodiments, the RNAi agent is administered with a buffer solution. In such embodiments, the buffer solution can comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. For example, the buffer solution can be phosphate buffered saline (PBS).

In an aspect, the invention provides a pharmaceutical composition comprising the double stranded RNAi agent of the invention and a lipid formulation. In certain embodiments, the lipid formulation comprises a LNP. In certain embodiments, the lipid formulation comprises MC3.

In an aspect, the invention provides a method of inhibiting IGFALS or IGF-1 expression in a cell, the method comprising (a) contacting the cell with the double stranded RNAi agent of the invention or a pharmaceutical composition of the invention; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of an IGFALS or IGF-1 gene, thereby inhibiting expression of the IGFALS or IGF-1 gene in the cell. In certain embodiments, the cell is within a subject, for example, a human subject, for example a female human or a male human. In preferred embodiments, IGFALS or IGF-1 expression is inhibited by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%, or to below the threshold of detection of the assay method used. Preferably the expression is inhibited by at least 50%. In some embodiments of the methods of the invention, expression of an IGF-1 gene is inhibited by at least 30%, 40%, 50%, 60%, 70%, 80%,

90%, or 95% of the difference between the elevated level associated with the disease and a normal level in an appropriate control subject. Preferably the elevated level is inhibited by at least 50%.

In an aspect, the invention provides a method of treating a subject having a disease or disorder that would benefit from reduction in IGFALS or IGF-1expression, such as an IGF system-associated disease or disorder, the method comprising administering to the subject a therapeutically effective amount of a double stranded RNAi agent of the invention or a pharmaceutical composition of the invention, thereby treating the subject.

In an aspect, the invention provides a method of preventing at least one symptom in a subject having a disease or disorder that would benefit from reduction in IGFALS or IGF-1expression, , such as an IGF system-associated disease or disorder, the method comprising administering to the subject a prophylactically effective amount of a double stranded RNAi agent of the invention or a pharmaceutical composition of the invention, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in IGFALS or IGF-1 expression.

In certain embodiments, the administration of the double stranded RNAi to the subject causes a decrease in the IGF-1signaling pathway. In certain embodiments, the administration of the double stranded RNAi causes a decrease in the level of IGF-1 or IGFALS in the subject, *e.g.*, serum levels of IGF-1 or IGFALS in the subject.

In certain embodiments, the IGF system-associated disease is acromegaly. In certain embodiments, the IGF system-associated disease is gigantism. In another embodiment, the IGF system-associated disease is cancer. In certain embodiments, the cancer is metastatic cancer.

In certain embodiments, the invention further comprises administering an inhibitor of growth hormone to a subject with an IGF system-associated disease.

In certain embodiments, the invention further comprises administering an inhibitor of the IGF pathway signaling to a subject with an IGF system-associated disease.

In certain embodiments, whereinthe IGF system-associated disease is acromegaly or gigantism, the subject is further treated for acromegaly or gigantism. In certain embodiments, the treatment for acromegaly or gigantism includes surgery. In certain embodiments, the treatment for acromegaly or gigantism includes radiation. In certain embodiments, the treatment for acromegaly or gigantism includes administration of a therapeutic agent.

In certain embodiments, wherein the IGF system-associated disease is cancer, the subject is further treated for cancer. In certain embodiments, the treatment for cancer includes surgery. In certain embodiments, the treatment for cancer includes radiation. In certain embodiments, the treatment for cancer includes administration of a chemotherapeutic agent.

In various embodiments, the dsRNAi agent is administered 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. In some embodiments, the dsRNAi agent is administered at a dose of about 10 mg/kg to about 30 mg/kg. In certain embodiments, the dsRNAi agent is administered at a dose selected from 0.5 mg/kg 1 mg/kg, 1.5 mg/kg, 3 mg/kg, 5 mg/kg, 10 mg/kg, and 30 mg/kg. In certain embodiments, the dsRNAi agent is administered about once per

week, once per month, once every other two months, or once a quarter (*i.e.*, once every three months) at a dose of about 0.1 mg/kg to about 5.0 mg/kg.

In certain embodiments, the double stranded RNAi agent is administered to the subject once a week. In certain embodiments, the dsRNAi agent is administered to the subject once a month. In certain embodiments, the dsRNAi agent is administered once per quarter (*i.e.*, every three months).

In some embodiment, the dsRNAi agent is administered to the subject subcutaneously.

In various embodiments, the methods of the invention further comprise determining the level of IGF-1 in the subject. In certain embodiments, a decrease in the level of expression or activity of the IGF-1 signaling pathway indicates that the IGF system-associated disease is being treated.

In various embodiments, a surrogate marker of IGF-1 expression is measured. In certain embodiments, a change, preferably a clinically relevant change in the surrogate marker indicating effective treatment of diseases associated with an elevated IGF-level are detected, *e.g.*, decreased serum IGF. In the treatment of acromegaly, a clinically relevant change in one or more signs or symptoms associated with acromegaly as provided below can be used as a surrogate marker for a reduction in IGF-1 expression. In the treatment of cancer, a demonstration of stabilization or reduction of tumor burden using RECIST criteria can be used as a surrogate marker for a reduction of IGF-1 expression or activity.

Brief Description of the Drawings

Figure 1 is a schematic showing various aspects of the IGF-1signaling pathways.

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 an Insulin-like Growth Factor Binding Protein, Acid Labile Subunit (IGFALS) or Insulin-like Growth Factor 1 (IGF-1) gene. The gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The use of these iRNAs enables the targeted degradation of mRNAs of the correponding gene (IGFALS or IGF-1 gene) in mammals.

The iRNAs of the invention have been designed to target a human IGFALS or a human IGF-1 gene, including portions of the gene that are conserved in the IGFALS or IGF-1 othologs of other mammalian species. Without intending to be limited by theory, it is believed that a combination or sub-combination of the foregoing properties and the specific target sites or the specific modifications in these iRNAs confer to the iRNAs of the invention improved efficacy, stability, potency, durability, and safety.

Accordingly, the present invention also provides methods for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of an IGFALS or IGF-1 gene, *e.g.*, an IGF system-associated disease, such as acromegaly or cancer, such as a cancer in which the tumor expresses IGF-1, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an IGFALS or an IGF-1 gene.

Very low dosages of the iRNAs of the invention, in particular, can specifically and efficiently mediate RNA interference (RNAi), resulting in significant inhibition of expression of the correponding target gene (IGFALS or IGF-1gene).

The iRNAs of the invention 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 an IGFALS or IGF-1 gene.

In certain embodiments, the iRNAs of the invention include an RNA strand (the antisense strand) which can include longer lengths, for example up to 66 nucleotides, *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 an IGFALS or an IGF-1 gene.

In some embodiments, the iRNA agents for use in the methods of the invention include an RNA strand (the antisense strand) which can be 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 an IGFALS or an IGF-1 gene. In some embodiments, such iRNA agents having longer length antisense strands preferably 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.

Using *in vitro* and *in vivo* assays, the present inventors have demonstrated that iRNAs targeting an IGFALS gene or an or IGF-1 gene can mediate RNAi, resulting in significant inhibition of expression of IGFALS or IGF-1, as well as reducing signaling through the IGF-1 pathway which will decrease one or more of the symptoms associated with an IGF system-associated disease, such as acromegaly or cancer. Thus, methods and compositions including these iRNAs are useful for treating a subject having an IGF system-associated disease, such as acromegaly or cancer. The methods and compositons herein are useful for reducing the level of IGFALS or IGF-1 in a subject, *e.g.*, serum or liver IGF-1 in a subject, especially in a subject with acromegaly or a tumor, such as an IGF-1 expressing tumor.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of an IGFALS gene or an IGF gene as well as compositions, uses, and methods for treating subjects having diseases and disorders that would benefit from reduction of the expression of an IGFALS gene or an IGF 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. For example, "sense strand or antisense strand" is understood as "sense strand or antisense strand or sense strand and antisense strand."

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 "at least" prior to a number or series of numbers is understood to include the number adjacent to the term "at least", and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 18 nucleotides of a 21 nucleotide nucleic acid molecule" means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range.

As used herein, "no more than" or "less than" is understood as the value adjacent to the phrase and logical lower values or intergers, as logical from context, to zero. For example, a duplex with an overhang of "no more than 2 nucleotides" has a 2, 1, or 0 nucleotide overhang. When "no more than" is present before a series of numbers or a range, it is understood that "no more than" can modify each of the numbers in the series or range.

As used herein, ranges include both the upper and lower limit.

In the event of a conflict between a sequence and its indicated site on a transcript or other sequence, the nucleotide sequence recited in the specification takes precedence.

Various embodiments of the invention can be combined as determined appropriate by one of skill in the art.

As used herein, "insulin-like growth factor binding protein, acid labile subunit" or "IGFALS" is a serum protein that binds insulin-like growth factors, increasing their half-life and their vascular localization. Production of the encoded protein, predominantly in the liver, which contains twenty leucine-rich repeats, is stimulated by growth hormone. Defects in this gene are a cause of acid-labile subunit deficiency, which manifests itself in delayed and slow puberty. Three transcript variants encoding two different isoforms have been found for this gene. The gene can also be known as ALS

or ACLSD. Further information on IGFALS is provided, for example in the NCBI Gene database at www.ncbi.nlm.nih.gov/gene/3483 (which is incorporated herein by reference as of the date of filing this application).

As used herein, "insulin-like growth factor binding protein, acid labile subunit," used interchangeably with the term "IGFALS," refers to the naturally occurring gene that encodes an IGF-1 binding protein. The amino acid and complete coding sequences of the reference sequence of the human IGFALS gene may be found in, for example, GenBank Accession No. GI: 225579150 (RefSeq Accession No. NM_004970.2; SEQ ID NO:1; SEQ ID NO:2), GenBank Accession No. GI:225579151 (RefSeq Accession No. NM_001146006.1; SEQ ID NO: 9 and 10). Mammalian orthologs of the human IGFALS gene may be found in, for example, GI:142388344 (RefSeq Accession No. NM_008340.3, mouse; SEQ ID NO:3 and SEQ ID NO:4); GI:71896591 (RefSeq Accession No. NM_053329.2, rat; SEQ ID NO:5 and SEQ ID NO:6); GenBank Accession Nos. GI:544514850 (RefSeq Accession No. XM_005590898.1, cynomolgus monkey; SEQ ID NO:7 and SEQ ID NO:8).

A number of naturally occurring SNPs are known and can be found, for example, in the SNP database at the NCBI at www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=3483 (which is incorporated herein by reference as of the date of filing this application) which lists SNPs in human IGFALS. In preferred embodiments, such naturally occuring variants are included within the scope of the IGFALS gene sequence.

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

"Insulin-like growth factor 1" or "IGF-1", also known as MGF, encodes a protein similar to insulin in function and structure and is a member of a family of proteins involved in mediating growth and development. The encoded protein is processed from a precursor, bound by a specific receptor, and secreted. Defects in this gene are a cause of insulin-like growth factor I deficiency. Alternative splicing results in multiple transcript variants encoding different isoforms that may undergo similar processing to generate mature protein.. Further information on IGF-1 is provided, for example, in the NCBI Gene database at www.ncbi.nlm.nih.gov/gene/3479 (which is incorporated herein by reference as of the date of filing this application).

As used herein, "insulin-like growth factor 1" is used interchangeably with the term "IGF-1" (and optionally any of the other recognized names listed above) refers to the naturally occurring gene that encodes an insulin-like growth factor 1 protein. The amino acid and complete coding sequences of the reference sequence of the human IGF-1 gene, transcript variant 1, mRNA, may be found in, for example, GenBank Accession No. GI: 930588898 (RefSeq Accession No. NM_001111283.2; SEQ ID NO: 11; SEQ ID NO: 12); human IGF-1 gene, transcript variant 4, mRNA, may be found at GenBank Accession No. GI: 930616505 (RefSeq Accession No. NM_000618.4; SEQ ID NO: 13 and SEQ ID NO:14); and human IGF-1, transcript variant 2, mRNA, may be found at GenBank Accession No. GI: 163659900 (RefSeq Accession No. NM_001111284.1; SEQ ID NO: 15 and 16. Mammalian

orthologs of the human IGF-1 gene may be found in, for example, GI: 930155588 (RefSeq Accession No. NM_010512.5, mouseIGF-1; SEQ ID NO:17 and SEQ ID NO:18); GI: 126722710 (RefSeq Accession No. NM_001082478.1, rat; SEQ ID NO:19 and SEQ ID NO:20); GenBank Accession Nos. GI: 544472486 (RefSeq Accession No. XM_005572040.1, cynomolgus monkey; SEQ ID NO:21 and SEQ ID NO:22). Multiple sequence variants for each of the species are known.

A number of naturally occurring SNPs are known and can be found, for example, in the SNP database at the NCBI at www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=3479 (which is incorporated herein by reference as of the date of filing this application) which lists SNPs in human IGF-1. In preferred embodiments, such naturally occuring variants are included within the scope of the IGF-1 gene sequence.

Additional examples of IGF-1 mRNA sequences are readily available using publicly available databases, *e.g.*, GenBank, UniProt, and OMIM.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an IGFALS gene or an IGF-1gene, including mRNA that is a product of RNA processing of a primary transcription product. 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 an IGFALS gene or an IGF-1gene gene. In one embodiment, the target sequence is within the protein coding region of IGFALS or IGF-1.

The target sequence 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. In some embodiments, the target sequence is about 19 to about 30 nucleotides in length. In other embodiments, the target sequence is about 19 to about 25 nucleotides in length. In still other embodiments, the target sequence is about 19 to about 23 nucleotides in length. In some embodiments, the target sequence is about 21 to about 23 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," and "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 the target gene, *e.g.,* an IGFALS gene or an IGF-1 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 RNAi that interacts with a target RNA sequence, *e.g.,* an IGFALS or IGF-1 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 double-stranded short interfering RNAs (siRNAs) comprising a sense strand and an antisense strand by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes these dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). These 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 (ssRNA) (the antisense strand of an siRNA duplex) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.,* an IGFALS or IGF-1 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 RNA that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents 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 RNAs 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 certain embodiments, an "iRNA" for use in the compositions, uses, 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.,* an IGFALS gene or an IGF-1 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 or a modified nucleotide. In addition, as used in this specification, an "iRNA" may include ribonucleotides with chemical modifications; an iRNA 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, or modified nucleobase, or any combination thereof. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.,* a functional group or atom, to internucleoside 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 "iRNA" or "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, 3, 4, 5, 6, 7, 8, 9, 10, 20, 23 or more unpaired nucleotides. In some embodiments, the hairpin loop can be 10 or fewer nucleotides. In some embodiments, the hairpin loop can be 8 or fewer unpaired nucleotides. In some embodiments, the hairpin loop can be 4-10 unpaired nucleotides. In some embodiments, the hairpin loop can be 4-8 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 of the RNAi agent, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In certain embodiments, an iRNA agent of the invention is a dsRNA, each strand of which comprises 19-23 nucleotides, that interacts with a target RNA sequence, *e.g.*, an IGFALS gene or an IGF-1 gene. Without wishing to be bound by theory, 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).

In some embodiments, an iRNA of the invention is a dsRNA of 24-30 nucleotides, or possibly even longer, *e.g.*, 25-35, 27-53, or 27-49 nucleotides, that interacts with a target RNA sequence, *e.g.*, an IGFALS target mRNA sequence or an IGF-1 target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory, 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).

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of a double strainded iRNA. 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 of the dsRNA, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.,* 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.,* 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.,* 0-3, 1-3, 2-4, 2-5, 4-10, 5-10, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. 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., 1-30 nucleotides, 2-30 nucleotides, 10-30 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 overhang is replaced with a nucleoside thiophosphate.

"Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the double stranded RNAi agent, *i.e.*, no nucleotide overhang. A "blunt ended" double stranded RNAi agent is double stranded over its entire length, *i.e.*, no nucleotide overhang at either end of the molecule. The

RNAi agents of the invention include RNAi agents with no nucleotide overhang at one end (*i.e.*, agents with one overhang and one blunt end) or with no nucleotide overhangs at either end.

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.,* an IGFALS or IGF-1 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.,* an IGFALS or IGF-1 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, 2, or 1 nucleotides of the 5'- or 3'-end of the iRNA. In some embodiments, a double stranded RNAi agent of the invention includes a nucleotide mismatch in the antisense strand. In some embodiments, a double stranded RNAi agent of the invention includes a nucleotide mismatch in the sense strand. In some embodiments, the nucleotide mismatch is, for example, within 5, 4, 3, 2, or 1 nucleotides from the 3'-end of the iRNA. In another embodiment, the nucleotide mismatch is, for example, in the 3'-terminal nucleotide 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, "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.

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 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 a double stranded RNAi 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 an IGFALS gene or an IGF-1 gene). For example, a polynucleotide is complementary to at least a part of an IGFALS or IGF-1 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding an IGFALS or IGF-1 gene.

Accordingly, in some embodiments, the sense strand polynucleotides and the antisense polynucleotides disclosed herein are fully complementary to the target IGFALS or IGF-1sequence.

In one embodiment, the antisense polynucleotides disclosed herein are fully complementary to the target IGFALS sequence. In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target IGFALS 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 any one of SEQ ID NOs:1, 3, 5, 7, or 9, or a fragment of any one of SEQ ID NOs:1, 3, 5, 7, or 9, 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 other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target IGFALS sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 3, 5, 6, 8, 12, or 14, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 3, 5, 6, 8, 12, or 14, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In one embodiment, an RNAi agent of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target IGFALS sequence and comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 3, 5, 6, 8, 12, or 14, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 3, 5, 6, 8, 12, or 14, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In one embodiment, the antisense polynucleotides disclosed herein are fully complementary to the target IGF-1 sequence. In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target IGF-1 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 any one of SEQ ID NOs:11, 13, 15, 17, 19, or 21, or a fragment of any one of SEQ ID NOs:11, 13, 15, 17, 19, or 21, 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 other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target IGF-1 sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 9, 11, 15, 17, 18, or 20, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 9, 11, 15, 17, 18, or 20, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In one embodiment, an RNAi agent of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target IGF-1 sequence and comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of

Tables 9, 11, 15, 17, 18, or 20, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 9, 11, 15, 17, 18, or 20, 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 an aspect of the invention, an agent for use in the methods and compositions of the invention is a single-stranded antisense oligonucleotide molecule that inhibits a target mRNA *via* an antisense inhibition mechanism. The single-stranded antisense oligonucleotide molecule is complementary to a sequence within the target mRNA. The single-stranded antisense oligonucleotides can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. *et al.*, (2002) *Mol Cancer Ther* 1:347-355. The single-stranded antisense oligonucleotide molecule may be about 14 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense oligonucleotide molecule may comprise a sequence that is at least 14, 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense sequences described herein.

The phrase "contacting a cell with an iRNA," such as a dsRNA, as used herein, includes contacting a cell by any possible means. Contacting a cell with an iRNA 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 iRNA may be put into physical contact with the cell by the individual performing the method, or alternatively, the iRNA 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 iRNA. Contacting a cell in vivo may be done, for example, by injecting the iRNA into or near the tissue where the cell is located, or by injecting the iRNA 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 iRNA may contain or be coupled to a ligand, *e.g.*, GalNAc3, that directs the iRNA 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 iRNA and subsequently transplanted into a subject.

In certain embodiments, 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 diffusion or active cellular processes, or by auxiliary agents or devices. Introducing an iRNA into a cell may be in vitro 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 US Patent Nos. 5,032,401 and 5,607,677, and US 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 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, US 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) that expresses the target gene, either endogenously or heterologously. It is understood that the sequence of the PHD gene must be sufficiently complementary to the antisense strand of the iRNA agent for the agent to be used in the indicated species. In certain embodiments, 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 an IGFALS gene or an IGF-1 gene expression or replication; a human at risk for a disease, disorder or condition that would benefit from reduction in IGFALS or IGF-1 gene expression; a human having a disease, disorder or condition that would benefit from reduction in IGFALS or IGF-1 gene expression; or human being treated for a disease, disorder or condition that would benefit from reduction in IGFALS or IGF-1 gene expression, as described herein. In some embodiments, the subject is a female human. In other embodiments, the subject is a male human.

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 IGFALS or IGF-1 gene expression or IGFALS or IGF-1 protein production, *e.g.*, acromegaly, cancer. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

The term "lower" or "reduce" in the context of the level of IGFALS or IGF-1 gene expression or IGFALS or IGF-1 protein production in a subject, or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or below the level of detection for the detection method. In certain embodiments, the decrease is down to a level accepted as within the range of normal for an individual without such disorder which can also be referred to as a normalization of a level. For example, lowering cholesterol to 180 mg/dl or lower would be considered to be within the range of normal for a subject. A subject having a cholesterol level of 230 mg/dl with a cholesterol level decreased to 210 mg/dl would have a cholesterol level that was decreased by 40% (230-210/230-180 = 20/50 = 40% reduction). In certain embodiments, the reduction is the normalization of the level of a sign or symptom of a disease, a reduction in the difference between the subject level of a sign of the disease and the normal level of the sign for the disease (e.g., the upper level of normal when the level must be reduced to reach a normal level, and the lower level of normal when the level must be increased to reach a normal level). In certain

embodiments, the methods include a clinically relevant inhibition of expression of IGFALS or IGF-1, *e.g.* as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of IGFALS or IGF-1.

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 an IGFALS gene or an IGF-1gene or production of an IGFALS or an IGF-1protein, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, *e.g.,* a symptom of IGFALS or IGF-1 gene expression, such as the presence of elevated levels of proteins in the IGF signaling pathway, *e.g.*, acromegaly or cancer. The failure to develop a disease, disorder or condition, or the reduction in the development of a symptom or comorbidity 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 or disease progression (*e.g.*, delayed cancer progression as determined using RECIST criteria) by days, weeks, months or years is considered effective prevention. Prevention may require the administration of more than one dose.

As used herein, the term "IGF system-associated disease," used interchangeable with the terms "insulin-like growth factor binding protein, acid labile subunit-associated disease," "IGFALS-associated disease," "IGF-associated disease," or "IGF-1-associated disease" is a disease or disorder that is caused by, or associated with IGFALS or IGF gene expression or IGFALS or IGF protein production. The term "IGF system-associated disease" includes a disease, disorder or condition that would benefit from a decrease in IGFALS or IGF-1 gene expression, replication, or protein activity. Non-limiting examples of IGF system-associated diseases include, for example, acromegaly, gigantism, and cancer, especially metastatic cancer.

In certain embodiments, an IGF system-associated disease-associated disease is acromegaly.

"Therapeutically effective amount," as used herein, is intended to include the amount of an iRNA that, when administered to a patient for treating a subject having acromealgy, cancer, or IGF system-associated disease, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating or maintaining the existing disease or one or more symptoms of disease or its related comorbidities). The "therapeutically effective amount" may vary depending on the iRNA, how it is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, stage of pathological processes mediated by IGFALS or IGF-1 gene expression, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated. Treatment may require the administration of more than one dose.

"Prophylactically effective amount," as used herein, is intended to include the amount of an iRNA that, when administered to a subject who does not yet experience or display symptoms of acromealgy, cancer, or other IGF system-associated disease-associated diseases, but who may be predisposed to an IGF system-associated disease-associated disease, is sufficient to prevent or delay the development or progression of the disease or one or more symptoms of the disease for a clinically significant period of time. The "prophylactically effective amount" may vary depending on the

iRNA, how it 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 "prophylacticaly effective amount" also includes an amount of an iRNA that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. iRNAs 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, 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 state, 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). A "sample derived from a subject" can refer to blood drawn from the subject, or plasma derived therefrom. In certain embodiments when detecting a level of IGF-1, a "sample" preferably refers to a tissue or body fluid from a subject in which IGF-1 is detectable prior to administration of an agent of the invention, e.g., a liver biopsy from a subject with a acromegaly, a tumor. In certain subjects, e.g., healthy subjects, the level of IGF-1 may not be detectable in a number of body fluids, cell types, and tissues.

I. iRNAs of the Invention

The present invention provides iRNAs which inhibit the expression of an IGFALS gene or an IGF-1 gene. In preferred embodiments, the iRNA includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of an IGFALS gene or an IGF-1 gene in a cell, such as a cell within a subject, *e.g.*, a mammal, such as a human having an IGF system-associated disease-associated disease, *e.g.*, acromeagly. The dsRNAi agent 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 an IGFALS gene or an IGF-1 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 IGFALS gene or the IGF-1 gene, the iRNA inhibits the expression of the IGFALS gene or the IGF-1 gene (*e.g.*, a human, a primate, a non-primate, or a bird IGFALS gene or IGF-1 gene) by at least 20%, preferably at least 30%, 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. In preferred embodiments, inhibiton of expression is deteremined by the qPCR method provided in the examples. For in vitro assessment of activity, percent inhibition is determined using the methods provided in Example 2 at a single dose at a 10 nM duplex final concentration. For *in vivo* studies, the level after treatment can be compared to, for example, an appropriate historical control or a pooled population sample control to determine the level of reduction, *e.g.*, when a baseline value is no available for the subject.

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 an IGFALS gene or IGF-1 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 about 15 to 30 base pairs in length, *e.g.,* 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 about 15 to 30 nucleotides in length, *e.g.,* 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 dsRNA is about 15 to 23 nucleotides in length, or about 25 to 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 in length may 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 about 36 base pairs, *e.g.*, 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 miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target IGFALS or IGF-1 gene 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.,* 1-4, 2-4, 1-3, 2-3, 1, 2, 3, or 4 nucleotides. dsRNAs having at least one nucleotide

overhang can have 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 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.

Double stranded RNAi 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. Simlarly, single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

In an aspect, a dsRNA of the invention for inhibing the expression of an IGFALS gene includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence. The sense strand is selected from the group of sequences provided in any one of Tables 3, 5, 6, 8, 12, and 14, and the corresponding antisense strand of the sense strand is selected from the group of sequences in any one of Tables 3, 5, 6, 8, 12, and 14. 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 an IGFALS gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in any one of Table 3, 5, 6, 8, 12, and 14, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Table 3, 5, 6, 8, 12, and 14. In certain embodiments, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In other embodiments, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

In an aspect, a dsRNA of the invention for inhibing the expression of an IGF-1 gene includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence. The sense strand is selected from the group of sequences provided in any one of Tables 9, 11, 15, 17, 18, and 20, and the corresponding antisense strand of the sense strand is selected from the group of sequences in any one of Tables 9, 11, 15, 17, 18, and 20. 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 an IGF-1 gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in any one of Table 9, 11, 15, 17, 18, and 20, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Table 9, 11, 15, 17, 18, and 20. In

certain embodiments, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In other embodiments, 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, 9, 12, 15, and 18 are not described as modified 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 in any one of Tables 3, 6, 9, 12, 15, and 18, or the sequences of any one ofTables 5, 8, 11, 14, 17, and 20 that are modified, or the sequences of any one ofTables 5, 8, 11, 14, 17, and 20 that are conjugated to a ligand. In other words, the invention encompasses dsRNAs of any one of Tables 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, and 20 which are un-modified, un-conjugated, modified, or conjugated, as described herein.

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.*, *EMBO* 2001, 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 in any one of Tables 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, and 20, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes having one of the sequences of Tables 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, and 20 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 of Tables 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, and 20, and differing in their ability to inhibit the expression of an IGFALS gene or an IGF-1 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 provided in Tables 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, and 20 identify a site(s) in an IGFALS transcript or IGF-1 transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within one of these sites. 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 in Tables 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, and 20 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in an IGFALS gene or an IGF-1 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 or provided herein) 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, for example, in Tables 3and 5 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, *e.g.*, in Tables 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, and 20, 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 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 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 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 an IGFALS gene or an IGF-1 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 an IGFALS gene or IGF-1 gene. Consideration of the efficacy of iRNAs with mismatches in inhibiting expression of an IGFALS gene or an IGF-1 gene is important, especially if the particular

region of complementarity in an IGFALS gene or an IGF-1 gene is known to have polymorphic sequence variation within the population.

II. Modified iRNAs of the Invention

In certain embodiments, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is unmodified, and does not comprise, *e.g.,* chemical modifications or conjugations known in the art and described herein. In other embodiments, 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 or substantially all of the nucleotides of an iRNA are modified, *i.e.*, not more than 5, 4, 3, 2, or 1 unmodified nucleotides are present in a strand of the iRNA.

The nucleic acids featured in the invention can be synthesized 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; 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 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.

Representative US Patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, US 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 US Patents that teach the preparation of the above oligonucleosides include, but are not limited to, US 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.

Suitable RNA mimetics are contemplated for use in iRNAs provided herein, 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 in which 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 US patents that teach the preparation of PNA compounds include, but are not limited to, US 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 US Patent No. 5,489,677, and

the amide backbones of the above-referenced US Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced US 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 US patents that teach the preparation of such modified sugar structures include, but are not limited to, US Patent 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 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 deoxy-

thymine (dT), 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 US 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.*, Angewandte Chemie, International Edition, 1991, 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 US 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 US 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.

The RNA of an iRNA 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).

In some embodiments, the 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).

The RNA of an iRNA 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'-CH_2-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′-(CH_2)—O-2′ (LNA); 4′-(CH_2)—S-2′; 4′-(CH_2)$_2$—O-2′ (ENA); 4′-CH(CH_3)—O-2′ (also referred to as "constrained ethyl" or "cEt") and 4′-CH(CH_2OCH_3)—O-2′ (and analogs thereof; see, *e.g.*, US Patent No. 7,399,845); 4′-C(CH_3)(CH_3)—O-2′ (and analogs thereof; see *e.g.*, US Patent No. 8,278,283); 4′-CH_2—N(OCH_3)-2′ (and analogs thereof; see *e.g.*, US Patent No. 8,278,425); 4′-CH_2—O—N(CH_3)-2′ (see, e.g.,US Patent Publication No. 2004/0171570); 4′-CH_2—N(R)—O-2′, wherein R is H, C1-C12 alkyl, or a protecting group (see, *e.g.*, US Patent No. 7,427,672); 4′-CH_2—C(H)(CH_3)-2′ (see, *e.g.*, Chattopadhyaya *et al., J. Org. Chem.*, 2009, 74, 118-134); and 4′-CH_2—C(=CH_2)-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 US Patents and US Patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: US 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).

The RNA of an iRNA 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'-$CH(CH_3)$-O-2' bridge. In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

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

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

Representative US 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.

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 the nucleotides 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 iRNA. Suitable phosphate mimics are disclosed in, for example US Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

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 WO2013/075035, the entire contents of each of which are incorporated herein by reference. WO2013/075035 provides motifs of three identical modifications on three consecutive nucleotides into a sense strand or antisense strand of a dsRNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the dsRNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense or antisense strand. The

dsRNAi agent may be optionally conjugated with a GalNAc derivative ligand, for instance on the sense strand.

More specifically, when the sense strand and antisense strand of the double stranded RNAi agent are completely modified to have one or more motifs of three identical modifications on three consecutive nucleotides at or near the cleavage site of at least one strand of a dsRNAi agent, the gene silencing acitivity of the dsRNAi agent was observed.

Accordingly, the invention provides double stranded RNAi agents capable of inhibiting the expression of a target gene (*i.e.*, IGFALS or IGF-1 gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may be, independently, 12-30 nucleotides in length. For example, each strand may independently be 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.

The sense strand and antisense strand typically form a duplex double stranded RNA ("dsRNA"), also referred to herein as "dsRNAi agent." The duplex region of a dsRNAi agent may be 12-30 nucleotide pairs in length. For example, the duplex region can be 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, 27, 28, 29, or 30 nucleotides in length.

In certain embodiments, the sense and antisense strands may be even longer. For example, in certain embodiments, the sense strand and the antisense strand are independently 25-35 nucleotides in length. In certain embodiments, each the sense and the antisense strand are independently 27-53 nucleotides in length, e.g., 27-49, 31-49, 33-49, 35-49, 37-49, and 39-49 nucleotides in length.
In certain embodiments, the dsRNAi agent may contain one or more overhang regions or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The overhang can be, independently, 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. In certain embodiments, at least one strand of the dsRNAi agent comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.,* 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the dsRNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.,* 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the dsRNAi agent comprise an overhang of at least 1 nucleotide.

In certain embodiments, the overhang regions can include extended overhang regions as provided above. 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 certain embodiments, the nucleotides in the overhang region of the dsRNAi agent can each independently be a modified or unmodified nucleotide including, but no limited to 2'-sugar modified, such as, 2'-F, 2'-O-methyl, 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 dsRNAi 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 some embodiments, the overhang is present at the 3'-end of the sense strand, antisense strand, or both strands. In some embodiments, this 3'-overhang is present in the antisense strand. In some embodiments, this 3'-overhang is present in the sense strand.

The dsRNAi 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'- end of the sense strand or, alternatively, at the 3'-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 dsRNAi agent 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 certain embodiments, the dsRNAi 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 other embodiments, the dsRNAi 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 other embodiments, the dsRNAi 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 certain embodiments, the dsRNAi 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 certain embodiments, every nucleotide in the sense strand and the antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In certain embodiments each residue is independently modified with a 2'-O-methyl or 3'-fluoro, *e.g.,* in an alternating motif. Optionally, the dsRNAi agent further comprises a ligand (preferably $GalNAc_3$).

In certain embodiments, the dsRNAi 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 certain embodiments, the dsRNAi agent comprises sense and antisense strands, wherein the dsRNAi 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 region which is at least 25 nucleotides in length, and the second strand is sufficiently complemenatary 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 dsRNAi 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 dsRNAi agent further comprises a ligand.

In certain embodiments, the sense strand of the dsRNAi 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 certain embodiments, the antisense strand of the dsRNAi 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 a dsRNAi agent having a duplex region of 17-23 nucleotides 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; the 10, 11, 12 positions; the 11, 12, 13 positions; the 12, 13, 14 positions; or the 13, 14, 15 positions of the antisense strand, the count starting from the first nucleotide from the 5'-end of the antisense strand, or, the count starting from the first 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 dsRNAi agent from the 5'-end.

The sense strand of the dsRNAi 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 some embodiments, the sense strand of the dsRNAi 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 adajacent 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 chemistries 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 dsRNAi agent may contain more than one motif 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 some embodiments, the wing modification on the sense strand or antisense strand of the dsRNAi 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 other embodiments, the wing modification on the sense strand or antisense strand of the dsRNAi 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 dsRNAi 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 dsRNAi 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 some embodiments, every nucleotide in the sense strand and antisense strand of the dsRNAi 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 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 dsRNAi agent or may only occur in a single strand region of a dsRNAi agent. For example, a phosphorothioate modification at a non-linking O position may only occur at one or both ends, 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 the ends. 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 some embodiments, each residue of the sense strand and antisense strand is independently modified with LNA, CRN, cET, UNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-deoxy, 2'-hydroxyl, or 2'-fluoro. The strands can contain more than one modification. In one embodiment, each residue of the sense strand and antisense strand is independently modified with 2'- O-methyl or 2'-fluoro.

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 certain embodiments, the N_a 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 some embodiments, the dsRNAi 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'to 3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 5' to 3'of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5' to 3' of the strand and the alternating motif in the antisenese strand may start with "BBAABBAA" from 5' to 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 some embodiments, the dsRNAi 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 or antisense strand interrupts the initial modification pattern present in the sense strand or antisense strand. This interruption of the modification pattern of the sense or antisense strand by introducing one or more motifs of three identical modifications on three consecutive nucleotides to the sense or antisense strand may enhance the gene silencing acitivty against the target gene.

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

The iRNA 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, 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 or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand 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-standed RNAi agent comprises 6-8phosphorothioate internucleotide linkages. In some embodiments, the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-end and two phosphorothioate internucleotide linkages at the 3'-end, and the sense strand comprises at least two phosphorothioate internucleotide linkages at either the 5'-end or the 3'-end.

In some embodiments, the dsRNAi agent 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, or the 5'end of the antisense strand.

In some embodiments, 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 dsRNAi 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 dsRNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mistmatch 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 certain embodiments, the dsRNAi 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 certain embodiments, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from 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 other embodiments, the nucleotide at the 3'-end of the sense strand is deoxy-thymine (dT) or the nucleotide at the 3'-end of the antisense strand is deoxy-thymine (dT). For example, there is a short sequence of deoxy-thymine nucleotides, for example, two dT nucleotides on the 3'-end of the sense, antisense strand, or both strands.

In certain embodiments, 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 some embodiments, the N_a or N_b comprises modifications of alternating pattern.

In some embodiments, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the dsRNAi 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 first nucleotide,

from the 5'-end; or optionally, the count starting at the first 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 (II):

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' (II)

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 some embodiments, the N_a' or N_b' comprises modifications of alternating pattern.

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

In certain embodiments, Y′Y′Y′ motif is all 2'-OMe modified nucleotides.

In certain embodiments, 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' (IIb);

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

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

When the antisense strand is represented by formula (IIb), 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 (IIc), 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 (IId), 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' (Ia).

When the antisense strand is represented as formula (IIa), 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 some embodiments, the sense strand of the dsRNAi 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 first nucleotide from the 5'-end, or optionally, the count starting at the first 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 some embodiments the antisense strand may contain Y′Y′Y′ motif occurring at positions 11, 12, 13 of the strand, the count starting from the first nucleotide from the 5'-end, or optionally, the count starting at the first 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 a antisense strand being represented by any one of formulas (IIa), (IIb), (IIc), and (IId), respectively.

Accordingly, the dsRNAi 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 iRNA duplex represented by formula (III):

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'

(III)

wherein:

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

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

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

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

wherein each n_p', n_p, n_q', and n_q, 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 an iRNA duplex include the formulas below:

5' n_p - N_a -Y Y Y -N_a-n_q 3'

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

(IIIa)

5' n_p -N_a - Y Y Y -N_b -Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-Y'Y'Y'-N_b'-Z'Z'Z'-$N_a'$$n_q'$ 5'

(IIIb)

5' n_p-N_a- X X X -N_b -Y Y Y - N_a-n_q 3'

3' n_p'-N_a'-X'X'X'-N_b'-Y'Y'Y'-N_a'-n_q' 5'

(IIIc)

5' n_p -N_a -X X X -N_b-Y Y Y -N_b- Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-X'X'X'-N_b'-Y'Y'Y'-N_b'-Z'Z'Z'-N_a-n_q' 5'

(IIId)

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

When the dsRNAi agent is represented by formula (IIIb), each N_b independently represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5, or 1-4 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the dsRNAi agent is represented as formula (IIIc), each N_b, 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.

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

Each of X, Y, and Z in formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) may be the same or different from each other.

When the dsRNAi agent is represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), 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 dsRNAi agent is represented by formula (IIIb) or (IIId), 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 dsRNAi agent is represented as formula (IIIc) or (IIId), 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 certain embodiments, 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 certain embodiments, when the dsRNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications. In other embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications and $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide a via phosphorothioate linkage. In yet other embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a monovalent, a bivalent or a trivalent branched linker (described below). In other embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , $n_p' > 0$ and at least one n_p' 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 monovalent, a bivalent or a trivalent branched linker.

In some embodiments, when the dsRNAi agent is represented by formula (IIIa), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , $n_p' > 0$ and at least one n_p' 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 monovalent, a bivalent or a trivalent branched linker.

In some embodiments, the dsRNAi agent is a multimer containing at least two duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), 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 some embodiments, the dsRNAi agent is a multimer containing three, four, five, six, or more duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), 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 dsRNAi agents represented by at least one of formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) are linked to each other at the 5' end, and one or both of the 3' ends, and are optionally conjugated to 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.

Various publications describe multimeric iRNAs that can be used in the methods of the invention. Such publications include US Patent No. 7,858,769, WO2007/091269, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520 the entire contents of each of which are hereby incorporated herein by reference.

As described in more detail below, the iRNA that contains conjugations of one or more carbohydrate moieties to an iRNA can optimize one or more properties of the iRNA. In many cases, the carbohydrate moiety will be attached to a modified subunit of the iRNA. For example, the ribose sugar of one or more ribonucleotide subunits of a iRNA 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, or 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 iRNA 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 a serinol backbone or diethanolamine backbone.

In certain embodiments, the iRNA for use in the methods of the invention for inhibiting the expression of an IGFALS gene is an agent selected from the agents listed in any one of Tables 3, 5, 6, 8, 12, and 14. These agents may further comprise a ligand. These agents may further comprise a ligand.

In certain embodiments, the iRNA for use in the methods of the invention for inhibiting the expression of an IGF-1 gene is an agent selected from the agents listed in any one of Tables 9, 11, 15, 17, 18, and 20. These agents may further comprise a ligand.

III. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves chemically linking to the iRNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the iRNA e.g., into a cell. For example, the ligand can be attached to the sense strand, antisense strand or both strands, at the 3'-end, 5'-end or both ends. For instance, the ligand may be conjugated to the sense strand. In preferred embodiments, the ligand is conjgated to the 3'-end of the sense strand. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, *Proc. Natl. Acid. Sci. USA*, 1989, 86: 6553-6556), cholic acid (Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 1994, 4:1053-1060). In certain embodiments, the modification can include a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306-309; Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 1993, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J*, 1991, 10:1111-1118; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327-330; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al.*, J. *Pharmacol. Exp. Ther.*, 1996, 277:923-937).

In certain embodiments, 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 do 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-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, monovalent or multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucoseamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, 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. In certain embodiments, ligands include monovalent or multivalent galactose. In certain embodiments, ligands include cholesterol.

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

In other embodiments, 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 crosslinked 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:24). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO:25) 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:26) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO:27) 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.*, glycosylated 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, an 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).

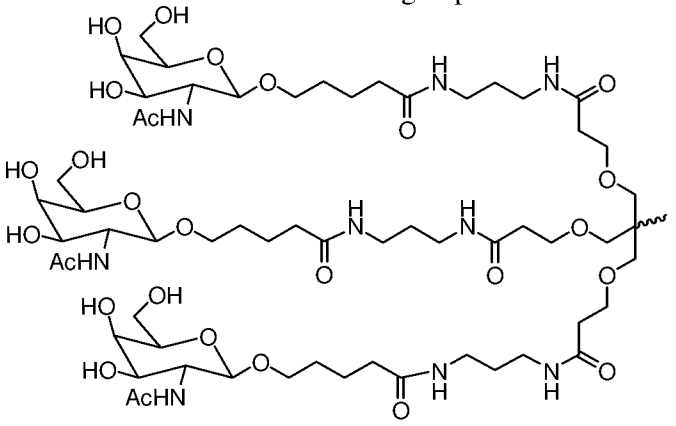
C. Carbohydrate Conjugates

In some embodiments of the compositions and methods of the invention, an iRNA further comprises a carbohydrate. The carbohydrate conjugated iRNA is 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 certain embodiments, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide.

In other embodiments, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group:



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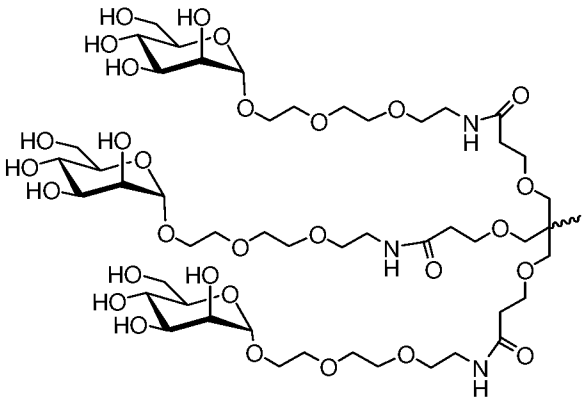

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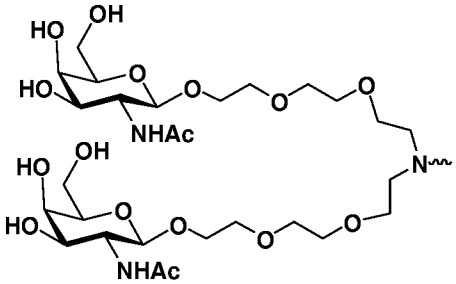

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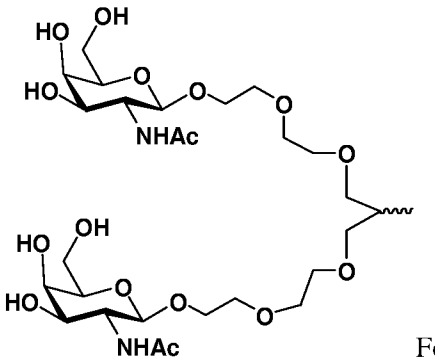

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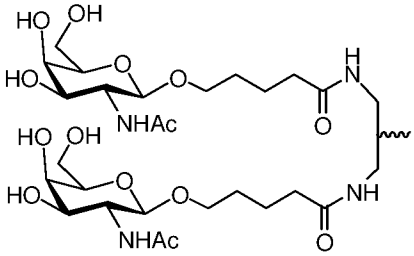

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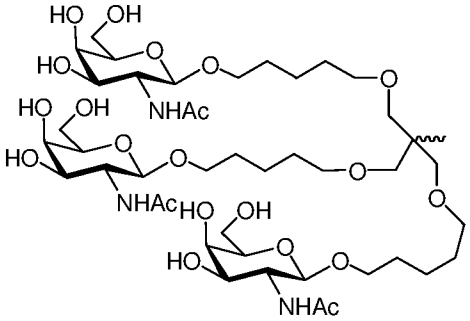

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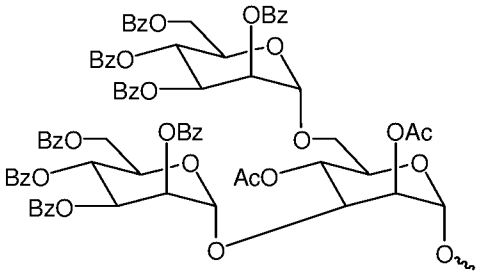

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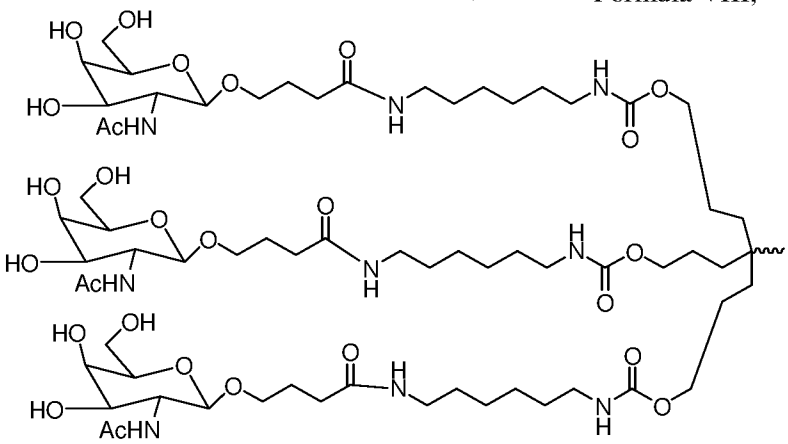

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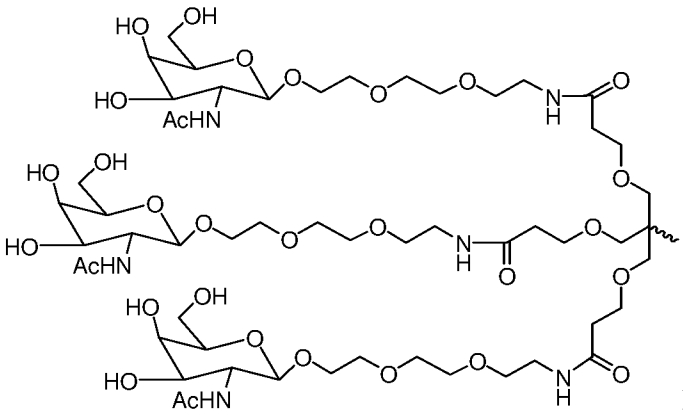

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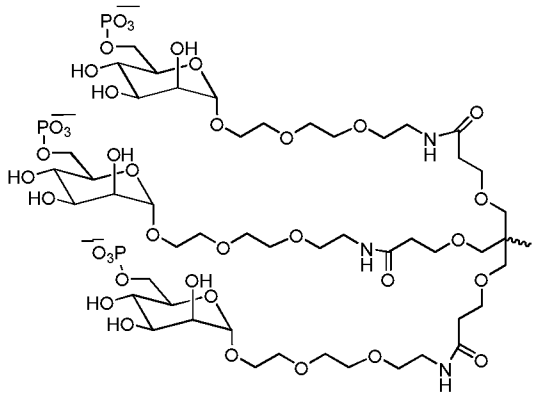

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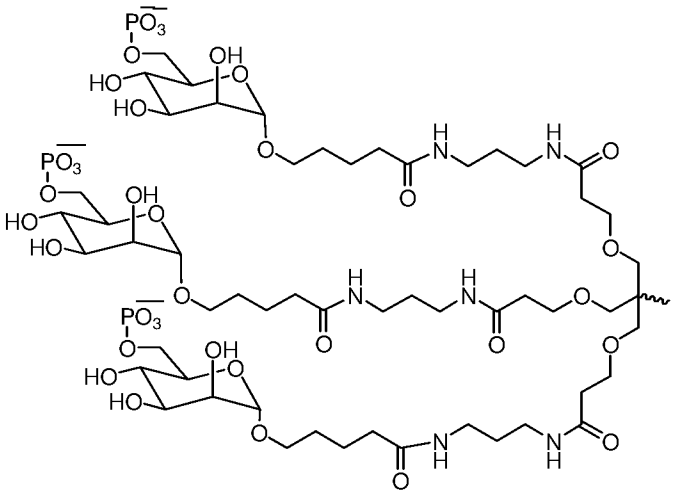

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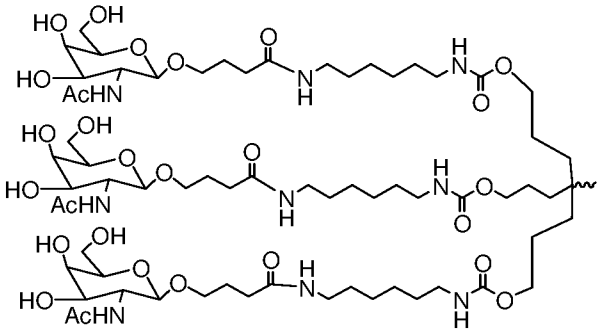

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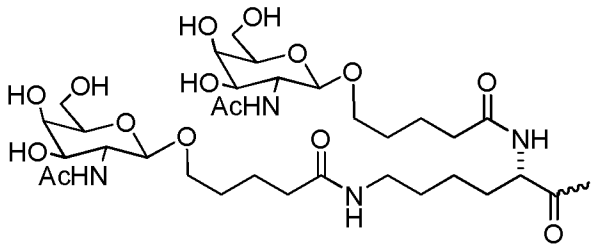

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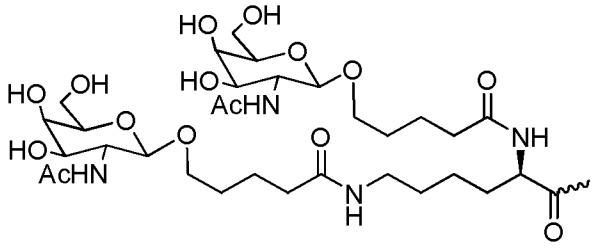

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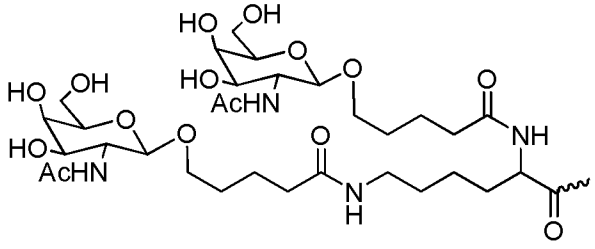

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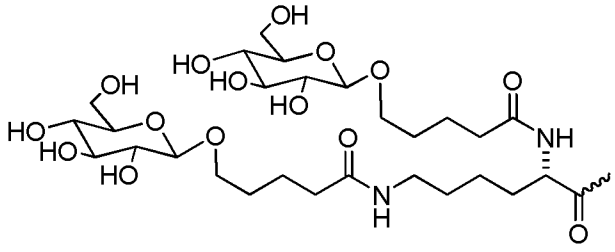

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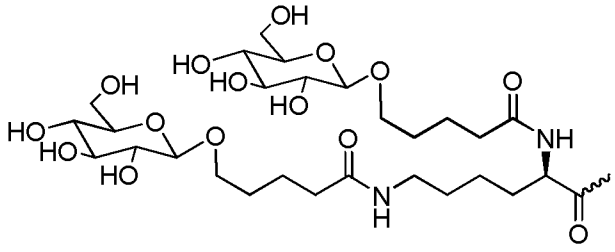

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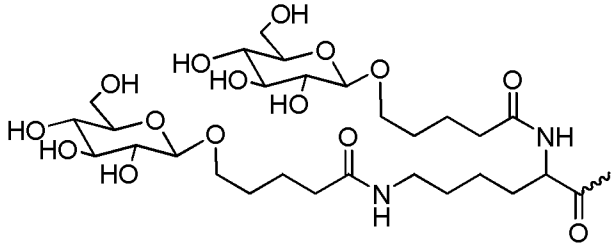

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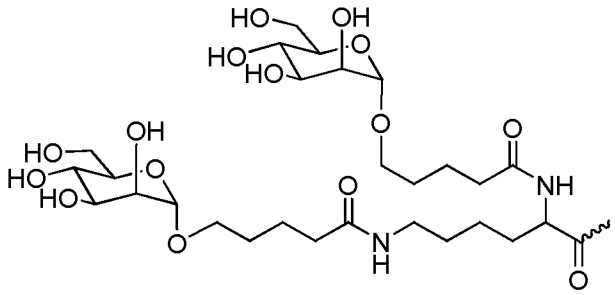
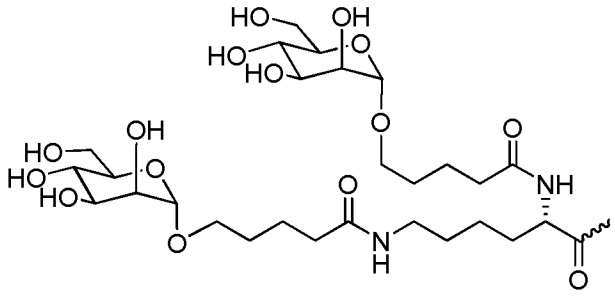
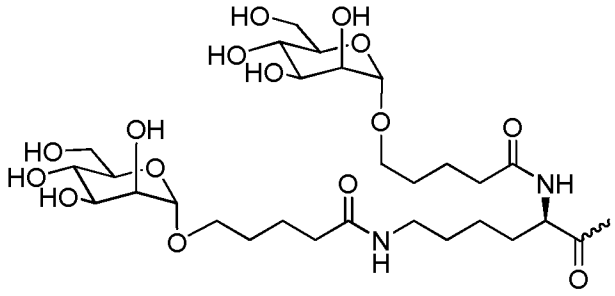


Formula XX,



Formula XXI,



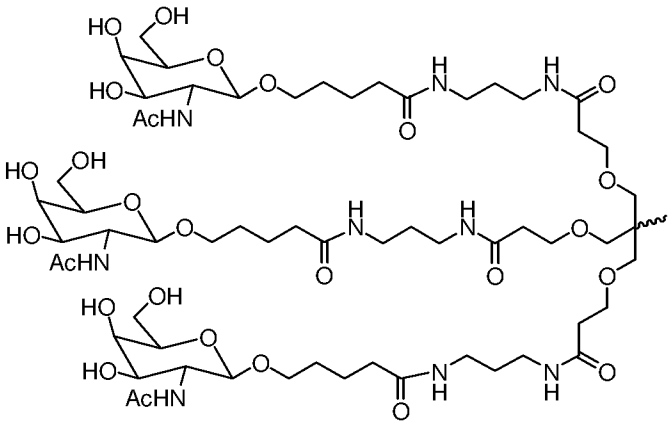
Formula XXII.

In certain embodiments, the ligand is an N-acetylgalactosamine (GalNAc) or GalNAc derivative. 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. In another embodiment, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

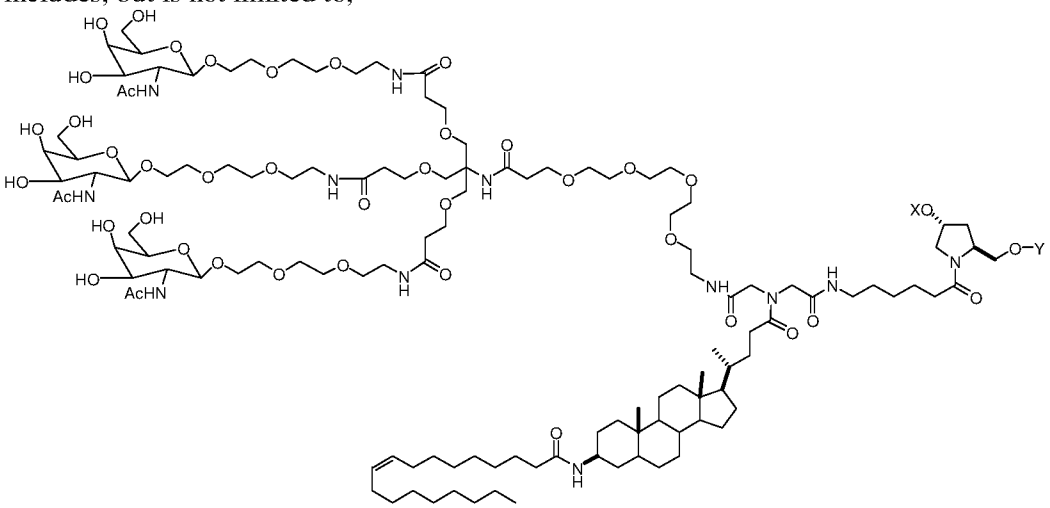
In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of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

The GalNAc or GalNAc derivative may be conjugated to the 3' end of the sense strand of the double stranded RNAi agent, the 5' end of the sense strand of the double stranded RNAi agent, the 3' end of the antisense strand of the double stranded RNAi agent, or the 5' end of the antisense strand of the double stranded RNAi agent. In certain embodiments, the monosaccharide is an N-acetylgalactosamine, such as



Formula I.

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



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

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 or a cell permeation peptide.

Additional carbohydrate conjugates 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, or 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, 7-17, 8-17, 6-16, 7-16, 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 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 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 certain embodiments, 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 other embodiments, 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-, and -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 other embodiments, 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, 5.5, 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.

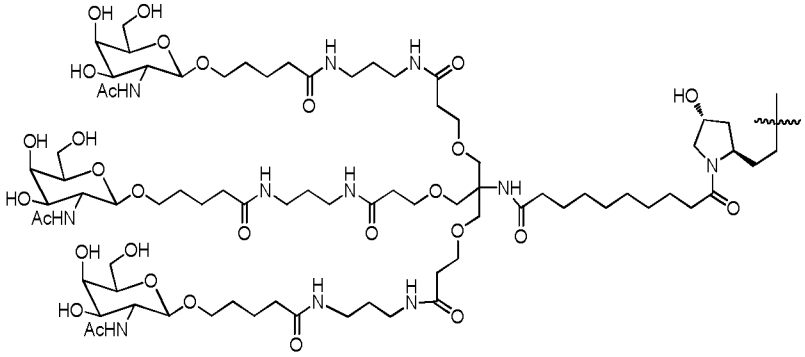
iv. Ester-based linking groups

In other embodiments, 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.

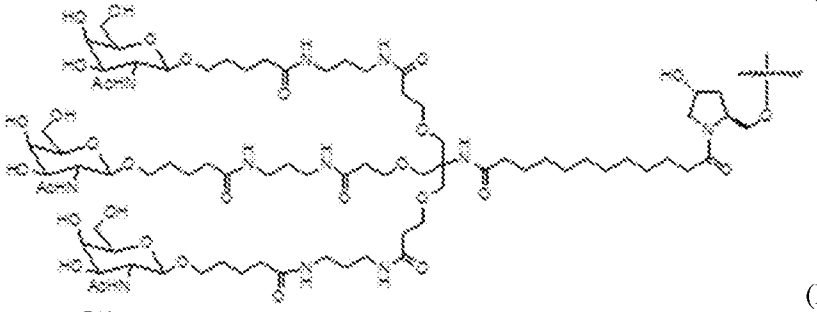
v. Peptide-based cleaving groups

In yet other embodiments, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides *etc.*) and polypeptides. Peptide-based cleavable groups do not include the amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or 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.

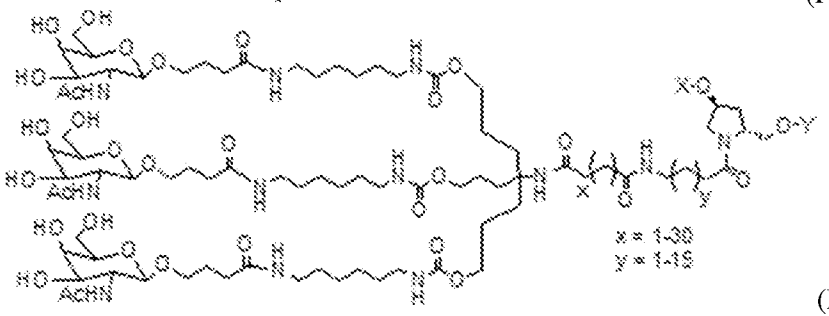
In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



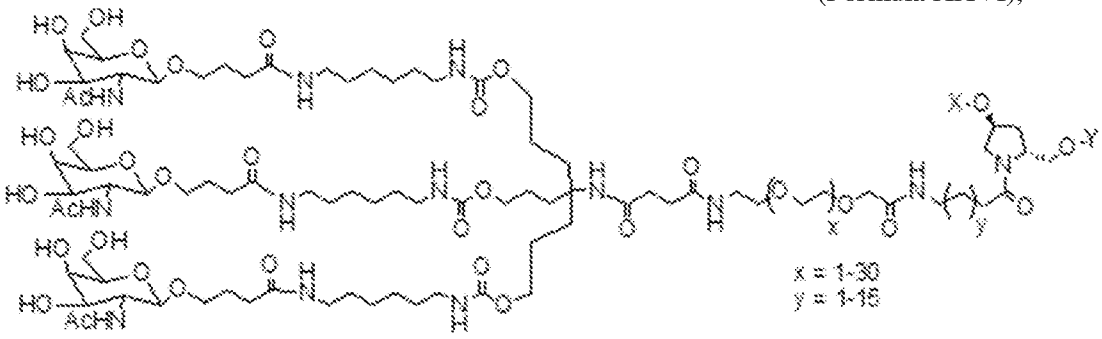
(Formula XXIV),



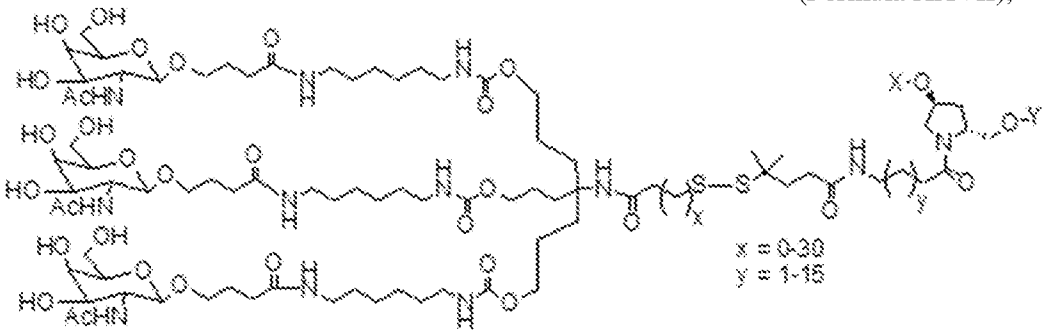
(Formula XXV),



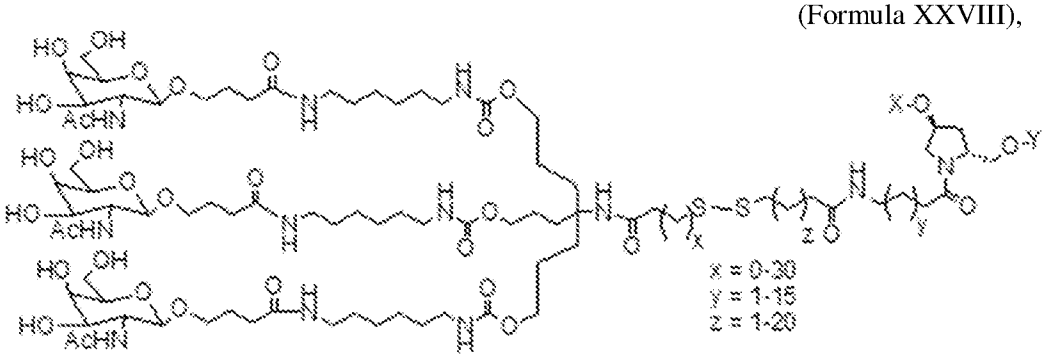
(Formula XXVI),



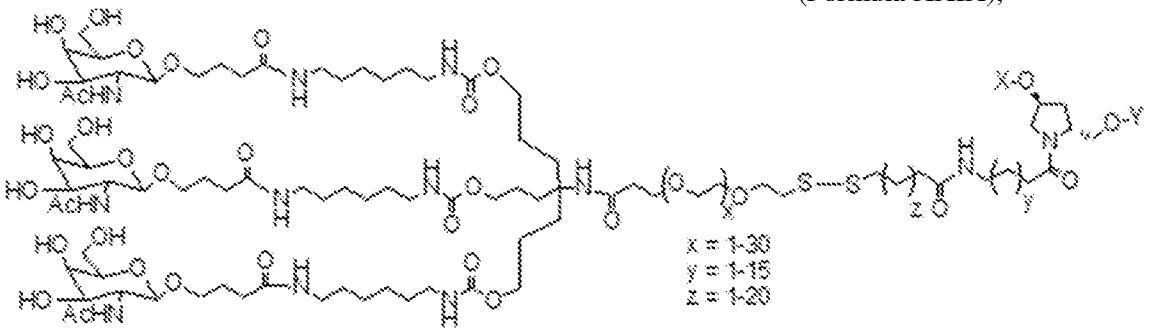
(Formula XXVII),



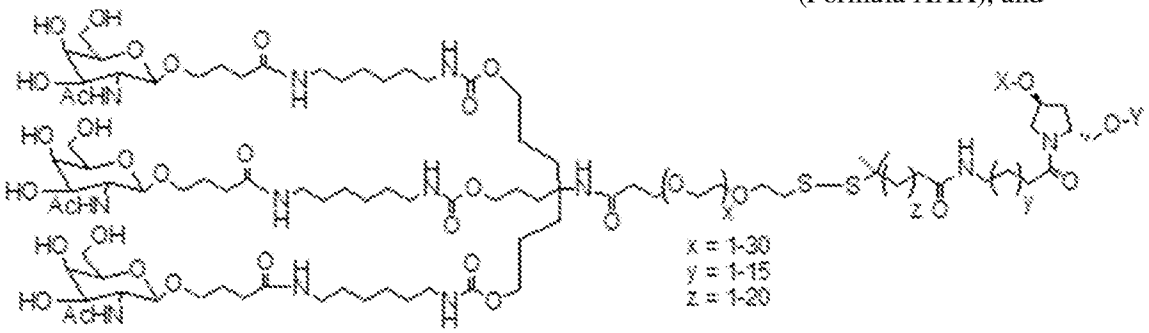
(Formula XXVIII),



(Formula XXIX),



(Formula XXX), and



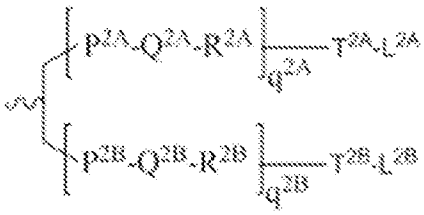
(Formula XXXI),

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

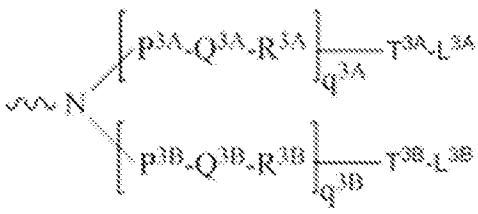
In certain embodiments of the compositions and methods of the invention, a ligand is one or more "GalNAc" (N-acetylgalactosamine) derivatives attached through a monovalent, a bivalent or a trivalent branched linker.

In certain embodiments, a dsRNA of the invention is conjugated to a monovalent, a bivalent or a trivalent branched linker selected from the group of structures shown in any of formula (XXXII) – (XXXV):

Formula XXXII



Formula XXXIII



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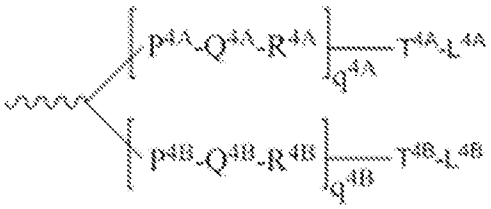

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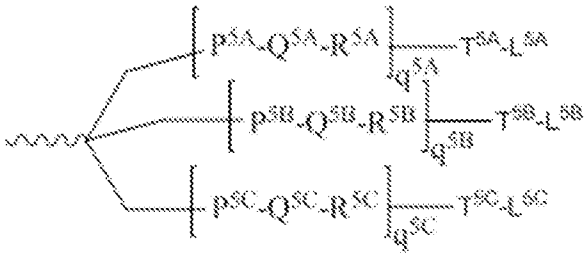


;

Formula XXXIV

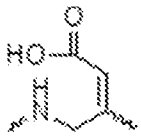
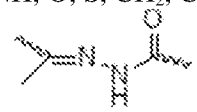
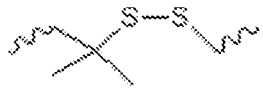
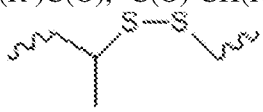
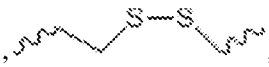
Formula XXXV

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;

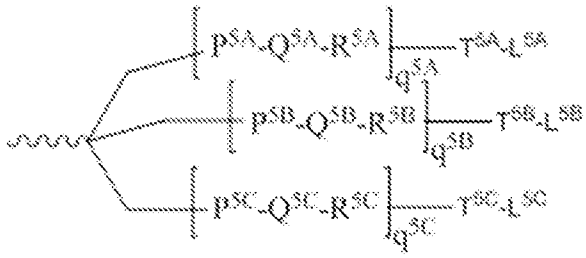
P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH, or CH_2O;

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 wherin 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; andR^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 (XXXV):

Formula XXXV



,

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

Examples of suitable monovalent, 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 US Patents that teach the preparation of RNA conjugates include, but are not limited to, US Patent 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;5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; and 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 dsRNAi agents, that 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, 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 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.

IV. 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 disease, disorder, or condition associated with IGFALS gene or IGF-1 gene expression) 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 a dsRNAi agent 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 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) J*ournal 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 US Patent No. 7,427,605, which is herein incorporated by reference in its entirety.

A. Vector encoded iRNAs of the Invention

iRNA targeting an IGFALS gene or an IGF-1 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, US Patent 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.*, *Proc. Natl. Acad. Sci. USA* (1995) 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.

V. Pharmaceutical Compositions of the Invention

The present invention also includes pharmaceutical compositions and formulations which include the iRNAs of the invention. In one embodiment, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA are useful for treating a disease or disorder associated with the expression or activity of an IGFALS gene or an IGF-1 gene. 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 subcutaneous (SC), intramuscular (IM), or intravenous (IV) delivery.

The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of an IGFALS gene or an IGF-1 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 regimine may include administration of a therapeutic amount of iRNA on a regular basis, such as every other day or once a year. In certain embodiments, the iRNA is administered about once per month to about once per quarter (i.e., about once every three months).

After an initial treatment regimen, the treatments can be administered on a less frequent basis. For example, after administration weekly or biweekly for three months, administration can be repeated once per month, for six months, or a year; or longer.

The pharmaceutical composition can be administered once daily, or the iRNA can be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even

using continuous infusion or delivery through a controlled release formulation. In that case, the iRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, *e.g.*, using a conventional sustained release formulation which provides sustained release of the iRNA over a several day period. Sustained release formulations are well known in the art and are particularly useful for delivery of agents at a particular site, such as could be used with the agents of the present invention. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

In other embodiments, a single dose of the pharmaceutical compositions can be long lasting, such that subsequent doses are administered at not more than 3, 4, or 5 day intervals, or at not more than 1, 2, 3, or 4 week intervals. In some embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered once per week. In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered bi-monthly.

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 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 known in the art. For example, a mouse model of acromegaly was developed by Kovacs *et al.* (1997, Endocrinology) the entire contents of which are incorporated herein by reference. Bovine growth hormone transgenic mice also exhibit features of acromegaly (Palmiter *et al.*, *Science* (1983), Olsson *et al.*, *Am J Phys Endo Metab* (2003), Berryman *et al, GH and IGF Res* (2004), Izzard *et al.*, *GH and IGF Res* (2009), Blutke *et al., Mol and Cell Endo* (2014)). Multiple animal models of cancer are known in the art.

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 tissue (*e.g.*, liver cells).

Pharmaceutical compositions and formulations for topical or transdermal 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 US 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. As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, *e.g.*, one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the iRNA. 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. 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 liposomal bilayer fuses with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the iRNA are delivered into the cell where the iRNA can specifically bind to a target RNA and can mediate RNA interference. In some cases the liposomes are also specifically targeted, *e.g.*, to direct the iRNA to particular cell types.

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 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, e*t 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 nucleic acid molecules to form a stable complex. The positively charged nucleic acid/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 nucleic acids rather than complex with it. Since both the nucleic acid and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some nucleic acid is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver nucleic acids 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 two or more of phospholipid, phosphatidylcholine, and 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 cyclosporine 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). US Patent 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. US Patent 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., P*roc. 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 can 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.,* dsRNAi agents of the invention may be fully encapsulated in a lipid formulation, *e.g.,* a 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). 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.*, US Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; US Publication No. 2010/0324120 and PCT Publication No. WO 96/40964.

In one embodiment, 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 can 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), l,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), l,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 can comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

In some embodiments, the compound 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane can be used to prepare lipid-siRNA nanoparticles.

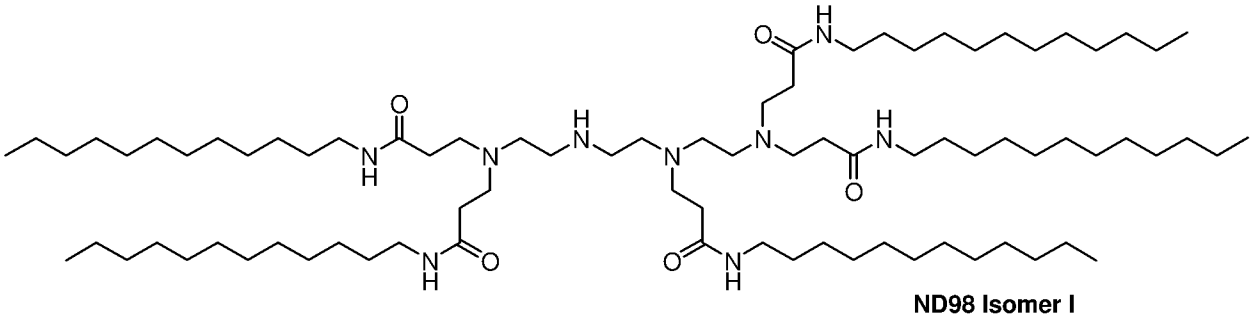
In some embodiments, the lipid-siRNA particle includes 40% 2, 2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane: 10% DSPC: 40% Cholesterol: 10% PEG-C-DOMG (mole percent) with a particle size of 63.0 ± 20 nm and a 0.027 siRNA/Lipid Ratio.

The ionizable/non-cationic lipid can 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 can 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 can 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 can be, for example, a PEG-dilauryloxypropyl (Ci_2), a PEG-dimyristyloxypropyl (Ci_4), a PEG-dipalmityloxypropyl (Ci_6), or a PEG- distearyloxypropyl ($C]_8$). The conjugated lipid that prevents aggregation of particles can 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.

In one embodiment, the lipidoid ND98·4HCl (MW 1487) (see US20090023673, which is incorporated herein by reference), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid-dsRNA nanoparticles (*i.e.*, 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 1

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 described in Table 1.

Table 1. Exemplary lipid formulations

	Ionizable/Cationic Lipid	**cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio**
SNALP-1	l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1
2-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

	Ionizable/Cationic Lipid	**cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio**
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 (Tech G1)	Tech G1/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP13	XTC	XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 33:1
LNP14	MC3	MC3/DSPC/Chol/PEG-DMG 40/15/40/5 Lipid:siRNA: 11:1
LNP15	MC3	MC3/DSPC/Chol/PEG-DSG/GalNAc-PEG-DSG 50/10/35/4.5/0.5 Lipid:siRNA: 11:1
LNP16	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP17	MC3	MC3/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP18	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 12:1
LNP19	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/35/5 Lipid:siRNA: 8:1

	Ionizable/Cationic Lipid	**cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio**
LNP20	MC3	MC3/DSPC/Chol/PEG-DPG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP21	C12-200	C12-200/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP22	XTC	XTC/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1

DSPC: distearoylphosphatidylcholine

DPPC: dipalmitoylphosphatidylcholine

PEG-DMG: PEG-didimyristoyl glycerol (C14-PEG, or PEG-C14) (PEG with avg mol wt of 2000)

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 International Application No. PCT/US2010/022614, filed January 29, 2010, which is hereby incorporated by reference.

MC3 comprising formulations are described, *e.g.*, in US Patent Publication No. 2010/0324120, filed June 10, 2010, the entire contents of 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 WO2010/129709, which is 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 or esters or salts thereof, bile acids 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 US Patent 6,887,906, US Publn. No. 20030027780, and US 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. Formulations include those that target the liver when treating hepatic disorders such as hepatic carcinoma.

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, or dextran. The suspension can also contain stabilizers.

C. Additional Formulations

i. Emulsions

The iRNAs 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 either in the 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 iRNAs 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 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., US 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., US 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 iRNA 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). Such compounds are well known in the art.

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, or dextran. The suspension can also contain stabilizers.

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA and (b) one or more agents which function by a non-iRNA mechanism and which are useful in treating an IGFALS or IGF-1-associated disorder.

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 LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased

concentration of the polypeptide) that includes the IC50 (*i.*e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the iRNAs featured in the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by IGFALS or IGF-1 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.

VI. Methods of the Invention

The present invention also provides methods of inhibiting expression of an IGFALS gene or IGF-1 gene in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, double stranded RNAi agent, in an amount effective to inhibit expression of IGFALS or IGF-1 in the cell, thereby inhibiting expression of IGFALS or IGF-1 in the cell.

Contacting of a cell with an iRNA, *e.g.*, a double stranded RNAi agent, may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the iRNA includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the iRNA. Combinations of *in vitro* and *in vivo* methods of contacting a cell are also possible. Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described herein or known in the art. In preferred embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a $GalNAc_3$ ligand, or any other ligand that directs the RNAi agent to a site of interest.

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 an IGFALS or "inhibiting expression of an IGF-1" is intended to refer to inhibition of expression of any IGFALS gene or IGF-1 gene (such as, *e.g.*, a mouse IGFALS gene or IGF-1 gene, a rat IGFALS gene or IGF-1 gene, a monkey IGFALS gene or IGF-1 gene, or a human IGFALS gene or IGF-1 gene) as well as variants or mutants of an IGFALS gene or IGF-1 gene. Thus, the IGFALS gene or IGF-1 gene may be a wild-type IGFALS gene or IGF-1 gene, a mutant IGFALS gene or IGF-1 gene (such as a mutant IGFALS gene or IGF-1 gene), or a transgenic IGFALS gene or IGF-1 gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of an IGFALS gene" or "inhibiting expression of an IGF-1 gene" includes any level of inhibition of an IGFALS gene or an IGF-1 gene, *e.g.*, at least partial suppression of the expression of an IGFALS gene or an IGF-1 gene. The expression of the IGFALS gene or an IGF-1 genemay be assessed based on the level, or the change in the level, of any variable associated with IGFALS gene or an IGF-1 geneexpression, *e.g.*, IGFALS mRNA or IGF-1 mRNA level or an

IGFALS protein level or an IGF-1 protein level. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with IGFALS or IGF-1 expression 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 some embodiments of the methods of the invention, expression of an IGFALS or IGF-1 gene is inhibited by at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In some embodiments, the inhibition of expression of an IGFALS gene or an IGF-1 gene results in normalization of the level of IGF-1 such that the difference between the level before treatment and a normal control level is reduced by at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%.

Inhibition of the expression of an IGFALS gene or an IGF-1 gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which an IGFALS gene or an IGF-1 gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with an iRNA of the invention, or by administering an iRNA of the invention to a subject in which the cells are or were present) such that the expression of an IGFALS gene or an IGF-1 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 not or have not been so treated (control cell(s) not treated with an iRNA or not treated with an iRNA targeted to the gene of interest). In preferred embodiments, the inhibition is assessed by the method provided in Example 2 and expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

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

In other embodiments, inhibition of the expression of an IGFALS gene or an IGF-1 gene may be assessed in terms of a reduction of a parameter that is functionally linked to IGFALS or IGF-1 gene expression, *e.g.*, IGFALS or IGF-1 protein expression or IGF signalinng pathways. IGFALS or IGF-1 gene silencing may be determined in any cell expressing IGFALS or IGF-1, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of an IGFALS or IGF-1 protein may be manifested by a reduction in the level of the IGFALS or IGF-1 protein that is expressed by a cell or group of cells (*e.g.*, the level of protein expressed in a sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibiton of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

A control cell or group of cells that may be used to assess the inhibition of the expression of an IGFALS or IGF-1 gene includes a cell or group of cells that has not yet been contacted with an RNAi agent of the invention. For example, the control cell or group of cells may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent.

In certain embodiments, inhibition of expression of an IGF-1 gene may be manifested in a reduction in the difference between a normal level of IGF-1 mRNA or protein and an abnormal level of IGF-1 mRNA or protein in a subject or in a specific tissue in the subject, e.g., mRNA in the liver of the subject or IGF-1 protein in subject serum. That is, inhibition may be manifested in a normalization of expression as compared to an appropriate control.

The level of IGFALS mRNA or IGF-1 mRNA that is expressed by a cell or group of cells, or the level of circulating IGFALS mRNA or IGF-1 mRNA, may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of IGFALS or IGF-1 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.,* mRNA of the IGFALS gene or IGF-1 gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy™ RNA preparation kits (Qiagen®) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays, northern blotting, *in situ* hybridization, and microarray analysis. Circulating IGFALS or IGF-1 mRNA may be detected using methods the described in PCT Publication WO2012/177906, the entire contents of which are hereby incorporated herein by reference.

In some embodiments, the level of expression of IGFALS or IGF-1 is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of selectively binding to a specific IGFALS or IGF-1. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to IGFALS mRNA or IGF-1 mRNA. In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of IGFALS mRNA or IGF-1 mRNA.

An alternative method for determining the level of expression of IGFALS or IGF-1 in a sample involves the process of nucleic acid amplification and/or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, *e.g.*, by RT-PCR (the experimental embodiment set forth in Mullis, 1987, US Patent No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi *et al.*, US Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of expression of IGFALS or IGF-1 is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System).

The expression levels of IGFALS or IGF-1 mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See US Patent Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of IGFALS or IGF-1 expression level may also comprise using nucleic acid probes in solution.

In preferred embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of these methods is described and exemplified in the Examples presented herein.

The level of IGFALS or IGF-1 protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like.

In some embodiments, the efficacy of the methods of the invention in the treatment of an IGF system-associated disease is assessed by a decrease in IGFALS mRNA or IGF-1 mRNA level (by liver biopsy) or IGFALS or IGF-1 protein level, typically determined in serum.

In some embodiments, the efficacy of the methods of the invention in the treatment of acromegaly can be monitored by evaluating a subject for normalization of at least one sign or symptom of acromegaly previously displayed in the subject including, elevated IGF-1 level, sleep apnea, joint pain, symptomatic carpal tunnel syndrome, hypertension, biventricular cardiac hypertrophy, cardiac arrhythmia, fatigue, and weakness. These symptoms may be assessed *in vitro* or

in vivo using any method known in the art. Although the nadir GH suppression ofter administration of glucose can be considered the "gold standard" test for acromegaly (Katznelson *et al.*, 2011, *Endocrine Practice*), suppression may not be observed after treatment with the RNAi agents provided herein due to their proposed mechanism of action. Moreover, subjects may have accomplished clinically relevant beneficial outcomes with lowering of IGF-1 without reaching normal GH levels.

It is understood that normal IGF-1 levels are dependent both on the age and gender of the subject, with younger subjects having lower IGF-1 levels than older subjects. Therefore, when comparing IGF-1 levels to determine the lowering or normalizing of the level, an appropriate control must be selected. Appropriate controls include, for example, an IGF-1 level prior to treatment (when available) or an age and gender matched control. In certain embodiments, IGF-1 levels are monitored or tested on multiple occasions to confirm a change in IGF-1 level in a subject. In preferred embodiments, the IGF-1 level is decreased sufficiently to provide a clinically beneficial outcome for the subject.

In some embodiments, the efficacy of the method of the invention in treatment of cancer can be monitored by evaluating a subject for maintenance or preferably reduction of tumor burden of the primary tumor or metastatic tumor(s) or the prevention of metastasis. Methods for detection and monitoring of tumor burden are known in the art, *e.g.*, RECIST criteria as provided in Eisenhauer *et al.*, 2009, New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur. J. Cancer. 45:228-247.

In some embodiments of the methods of the invention, the iRNA is administered to a subject such that the iRNA is delivered to a specific site within the subject. The inhibition of expression of IGFALS or IGF-1 may be assessed using measurements of the level or change in the level of IGFALS or IGF-1 mRNA or IGFALS or IGF-1 protein in a sample derived from fluid or tissue from the specific site within the subject.

As used herein, the terms detecting or determining a level of an anlyte are understood to mean performing the steps to determine if a material, *e.g.*, protein, RNA, is present. As used herein, methods of detecting or determining include detection or determination of an anlyte level that is below the level of detection for the method used.

VII. Methods of Treating or Preventing IGF System-Associated Diseases

The present invention also provides methods of using an iRNA of the invention or a composition containing an iRNA of the invention to reduce or inhibit IGFALS or IGF-1 expression in a cell. The methods include contacting the cell with a dsRNA of the invention and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of an IGFALS gene or an IGF-1 gene, thereby inhibiting expression of the IGFALS gene or an IGF-1 gene in the cell. Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of IGFALS or IGF-1 may be determined by determining the mRNA expression level of IGFALS or IGF-1, *e.g.*, in a liver sample, using methods routine to one of ordinary skill in the art,

e.g., northern blotting, qRT-PCR; by determining the protein level of IGFALS or IGF-1 using methods routine to one of ordinary skill in the art, such as western blotting, immunological techniques. A reduction in the expression of IGFALS or IGF-1 may also be assessed indirectly by measuring a decrease in biological activity of IGFALS or IGF-1 or measuring the level of IGF-1 in a subject sample (*e.g.*, a serum sample).

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 an IGFALS or IGF-1 gene, typically a liver cell. 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.

IGFALS expression or IGF-1expression is inhibited in the cell by at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to a level below the level of detection of the assay. IGFALS expression or IGF-1expression is inhibited in the cell such that the difference between the level of expression in a subject with an IGF system-associated disease and the normal level of expression is reduce by at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%.

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 IGFALS gene or IGF-1 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 IGFALS or IGF-1, 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.

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 an IGFALS or IGF-1 gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets an IGFALSor an IGF-1 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the IGFALS gene or the IGF-1 gene, thereby inhibiting expression of the IGFALS gene or the IGF-1 gene in the cell. 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, described herein. In one embodiment, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in the IGFALS gene or the IGF-1 gene or protein expression.

The present invention further provides methods of treatment of a subject in need thereof. The treatment methods of the invention include administering an iRNA of the invention to a subject, *e.g.*, a subject that would benefit from a reduction or inhibition of IGFALS or IGF-1 expression, in a therapeutically effective amount of an iRNA targeting an IGFALS gene or an IGF-1 gene or a pharmaceutical composition comprising an iRNA targeting an IGFALS gene or an IGF-1 gene.

An iRNA of the invention may be administered as a "free iRNA." A free iRNA is administered in the absence of a pharmaceutical composition. The naked iRNA may be 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.

Subjects that would benefit from a reduction or inhibition of IGFALS gene or an IGF-1 gene expression are those having a disorder of elevated growth hormone, *e.g.*, acromegaly, or a disorder of elevated insulin signaling, *e.g.*, cancer. In another embodiment, a subject having a disorder of elevated growth hormone has one or more signs or symptoms associated with acromegaly or elevated growth hormone including, but not limited to, elevated IGF-1 level, somatic enlargement (soft tissue and bony overgrowth), excessive sweating, jaw overgrowth, sleep apnea, osteoarthropathy, joint pain,

symptomatic carpal tunnel syndrome, hypertension, biventricular cardiac hypertrophy, cardiac arrhythmia, fatigue, weakness, diabetes mellitus, menstrual irregularities in women and sexual dysfunction in men, headache, and visual field loss (attributable to optic chiasmal compression) and diplopia (due to cranial nerve palsy); in conjunction with an elevated growth hormone level. Treatment of a subject that would benefit from a reduction or inhibition of IGFALS or IGF-1 gene expression and normalization of growth hormone levels includes therapeutic treatment (*e.g.,* of a subject is suffering from acromegaly) and prophylactic treatment (*e.g.,* of a subject does not meet the diagnostic criteria of acromegaly or may have elevated or fluctuating growth hormone, or IGFALS, or IGF-1 levels, or a subject may be at risk of developing acromegaly). Treatment of a subject that would benefit from a reduction or inhibition of IGFALS gene expression or IGF-1 gene expression can also include treatment of cancer.

The invention further provides methods for the use of an iRNA or a pharmaceutical composition thereof, *e.g.*, for treating a subject that would benefit from reduction or inhibition of IGFALS or IGF-1 expression, *e.g.,* a subject having a disorder of elevated growth hormone, in combination with other pharmaceuticals or other therapeutic methods, *e.g.*, with known pharmaceuticals or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders. For example, in certain embodiments, an iRNA targeting IGFALS or IGF-1 is administered in combination with an agent useful in treating a disorder of elevated growth hormone as described elsewhere herein.

The invention provides methods for the treatment of cancer, *e.g.*, IGF-1 dependent cancer, IGF-1 receptor positive cancer, or metastatic or potentially metastatic cancer. In certain embodiments, the iRNAs of the invention are used in conjunction with various standards of treatment of cancer, *e.g.*, chemotherapeutic agents, surgery, radiation; and combinations thereof.

The iRNA and additional therapeutic agents may be administered at the same time or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times or by another method known in the art or described herein.

In one embodiment, the method includes administering a composition featured herein such that expression of the target IGFALS gene or IGF-1 gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 18, 24 hours, 28, 32, or abour 36 hours. In one embodiment, expression of the target IGFALS gene or IGF-1 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 IGFALS gene or IGF-1 gene. Compositions and methods for inhibiting the expression of these genes using iRNAs can be prepared and performed as described herein.

Administration of the iRNA according to the methods of the invention may result in a reduction of the severity, signs, symptoms, or markers of such diseases or disorders in a patient with a disorder of elevated growth hormone, elevated IGFALS, elevated IGF-1, or an IGF-1 responsive

tumor. By "reduction" in this context is meant a statistically significant decrease in such level. The reduction can be, for example, at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay used.

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 IGF signaling may be assessed, for example, by periodic monitoring of IGF-1 or IGFALS levels, *e.g.*, serum IGF-1 or IGFALS levels. For subjects suffering from acromegaly a decrease in one or more signs or symptoms including, but not limited to sleep apnea, joint pain, symptomatic carpal tunnel syndrome, hypertension, biventricular cardiac hypertrophy, cardiac arrhythmia, fatigue, and weakness can be an indication of treatment of acromegaly. Similarly a delay or lessening of the severity of the co-morbidities associated with acromegaly such as hypertension, hypertrophy, stroke, diabetes, and sleep apnea can demonstrate efficacy of treatment.

Efficacy of treatment of cancer can be demonstrated by stabilization or a decrease in tumor burden as demonstrated by a stabilization or decrease in tumor burden of the primary tumor, metastatic tumors, or the delay or prevention of tumor metastasis. Diagnostic and monitoring methods are known in the art and are also provided herein.

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 targeting IGFALS or IGF-1, or pharmaceutical composition thereof, "effective against" an IGF system-associated disorder indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as a 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 IGF system-associated disorders.

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. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

Alternatively, the efficacy can be measured by a reduction in the severity of disease as determined by one skilled in the art of diagnosis based on a clinically accepted disease severity grading scale. Any positive change resulting in *e.g.*, lessening of severity of disease measured using the appropriate scale, represents adequate treatment using an iRNA or iRNA formulation as described herein.

Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 200 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 IGFALS or IGF-1 levels, *e.g.*, in a cell, tissue, blood, urine, or other compartment of the patient by at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or below the level of detection of the assay method used. It is noted that a reduction in IGFALS will not likely result in a decrease in growth hormone levels in a subject with acromegaly. Administration of the iRNA can reduce the difference in the subject IGF-1 levels and a normal IGF-1 level, *e.g.*, in a cell, tissue, blood, urine, or other compartment of the patient by at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%.

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 month to about once per quarter (*i.e.*, about once every three months).

IX. Diagnostic Criteria and Treatment for Acromegaly

Diagnostic criteria for acromegaly are set forth in the American Association of Clinical Endocrinologists Medical Guidelines for Clinical Practice for the Diagnosis and Treatment of Acromegaly – 2011 Update (Katznelson *et al.*, Endocr. Pract. 17(Suppl. 4), incorporated herein by reference. Further details and citations can be found therein.

Acromegaly is a clinical syndrome that, depending on its stage of progression, may not manifest with clear diagnostic features. Diagnosis should be considered in patients with 2 or more of the following comorbidities: new-onset diabetes, diffuse arthralgias, new-onset or difficult-to-control

hypertension, cardiac disease including biventricular hypertrophy and diastolic or systolic dysfunction, fatigue, headaches, carpal tunnel syndrome, sleep apnea syndrome, diaphoresis, loss of vision, colon polyps, and progressive jaw malocclusion. A serum IGF-I level, if accompanied by a large number of results from age- and sex-matched normal subjects, is a good tool to assess integrated GH secretion and is excellent for diagnosis, monitoring, and especially screening. A random IGF-I value (a marker of integrated GH secretion) should be measured for diagnosis and for monitoring after a therapeutic intervention. Serum GH assays are not standardized and should not be used interchangably. The nadir GH suppression after administration of glucose has been considered the "gold standard" test for acromegaly, however, a conflict exists regarding the threshold for diagnosis. The panel recommends that GH measurements be performed at baseline, then every 30 minutes for a total of 120 minutes after administration of glucose. The inability to suppress serum GH to less than 1 ng/mL after glucose administration is typically considered the diagnostic criterion for acromegaly, however, in a consensus guideline in 2000, the diagnosis of acromegaly was excluded if the patient had a random GH measurement less than 0.4 ng/mL and a normal IGF-I value. Although a nadir GH concentration of less than 1 ng/mL after administration of glucose is the standard recommendation for a normal response, the 2011 panel suggests consideration of a lower nadir GH cut point at 0.4 ng/mL after glucose administration because of the enhanced assay sensitivity and more frequent finding of modest GH hypersecretion. A diagnosis of acromegaly will be made by one of skill in the art considering the totality of the evidence for the patient under consideration.

Once a biochemical diagnosis of acromegaly has been made, a magnetic resonance imaging (MRI) scan of the pituitary gland should be performed because a pituitary GH-secreting adenoma is the most common cause of acromegaly. Visual field testing should be performed if there is optic chiasmal compression noted on the MRI or if the patient has complaints of reduced peripheral vision. Further biochemical testing should include a serum prolactin level (to evaluate for hyperprolactinemia) and assessment of anterior and posterior pituitary function (for potential hypopituitarism).

The goals of therapy for acromegaly are to (1) control biochemical indices of activity, (2) control tumor size and prevent local mass effects, (3) reduce signs and symptoms of disease, (4) prevent or improve medical comorbidities, and (5) prevent early mortality. The primary mode of therapy is surgery, which is recommended for all patients with microadenomas and for all patients who have macroadenomas with associated mass effects. In patients with macroadenomas without mass effects, and with low likelihood of surgical cure, a role for surgical de-bulking of macroadenomas to improve the response to subsequent medical therapy has been advocated, as well as primary medical therapy alone. Medical therapy is generally used in the adjuvant setting. Irradiation, either conventional fractionated RT or stereotactic radiosurgery, is largely relegated to an adjuvant role. Availability of specific therapeutic options and cost of these interventions are taken into account with decisions regarding therapy.

The goal of surgical interventions is to decrease tumor volume, thereby decreasing production of excess growth hormone and decompress the mass effect of macroadenomas on any normal remaining pituitary gland tissues, optic nerve, or surrounding critical structures. Surgical interventions can be curative for many subjects. Surgically resected tissue should be analyzed to understand the tumor biology to potentially provide guidance for treatment. Biochemical analyses are also performed post-operatively to assess the surgical outcome.

Medical therapy is used in conjunction with surgery. Studies have provided conflicting results regarding the benefits of treatment with medical interventions prior to surgery to change the nature of the tumor. The iRNAs provided herein can be used at any time in conjunction with surgical intervention (*i.e.*, before or after surgery).

Adjunctive medical therapy is used in patients who cannot achieve a complete cure by surgical intervention. Medical therapies fall into three catagories: dopamine agonists, somatostatin analogs (SSAs), and a GH receptor antagonist. Each of the medical interventions presents different risks and benefits, including substantial costs of some of the therapies.

Dopamine agonists include cabergoline and bromocriptine. The agents are a good first line therapy, especially in patients with mild biochemical activity, as they are realtively inexpensive and orally administered. However, side effects include gastrointestinal upset, orthostatic hypotension, headache, and nasal congestion.

Somatostatin analogs (SSAs) include octreotide (Sandostatin®) LAR (long-acting release, administered as an intramuscular injection) and lanreotide (Somatuline®) Autogel (administered as a deep subcutaneous depot injection). SSAs are less convenient for use than dopamine agonists as they must be administered by injection (50 mcg three times daily Sandostatin® Injection subcutaneously for 2 weeks followed by Sandostatin® LAR 20 mg intragluteally every 4 weeks for 3 months; or 60, 90, or 120mg of Somatuline® every 28 days by deep subcutaneous injection). SSAs are effective in normalizing IGF-I and GH levels in approximately 55% of patients. The clinical and biochemical responses to SSAs are inversely related to tumor size and degree of GH hypersecretion. Octreotide LAR and lanreotide Autogel have similar efficacy profiles. In patients with an inadequate response to SSAs, the addition of cabergoline or pegvisomant (Somavert®) may be effective for further lowering one or both of GH and IGF-1 levels. Potential side effects of SSAs, include gastrointestinal upset, malabsorption, constipation, gallbladder disease, hair loss, and bradycardia.

Pegvisomant, a GH receptor antagonist, is administered by daily subcutaneous injection. Side effects of pegvisomant, include flulike illness, allergic reactions, and increase in liver enzymes. Patients treated with pegvisomant must undergo routine liver enzyme tests. Because endogenous GH levels increase with pegvisomant administration and pegvisomant may be cross-measured in GH assays, serum GH levels are not specific and should not be monitored in patients receiving pegvisomant. Instead, serum IGF-1 levels are monitored.

Combinations of various medical therapies may be useful in the treatment of some acromegaly patients.

Radiation therapy is used as an adjunctive treatment is patients who do not respond sufficiently to surgical or medical interventions.

Similar treatment strategies are used in children with gigantism, a type of acromegaly, which refers to excess GH secretion that occurs during childhood when the growth plates are open, leading to accelerated vertical growth.

Some of the comorbidities of acromegaly resolve upon decreasing the level of GH or decreasing the responsiveness of the subject to GH. However, others are not. Unlike soft tissue changes, bone enlargement is not reversible. Surgical interventions (*e.g.*, carpal tunnel release, joint replacement surgery), physical therapy, and analgesic medications can be used to treat conditions associated with bone or soft tissue overgrowth. Respiratory disorders including sleep apnea and higher susceptibility to respiratory infections can be treated with standard interventions and preventive strategies (*e.g.*, influenza and pneumococcal vaccinations). Cardiovascular disease, hypertension, and stroke can be managed using standard monitoring (*e.g.*, blood pressure, cholesterol, and lipid level monitoring) and medical treatment. Subjects should be monitored for the development of type 2 diabetes and neoplasia, particularly colon polyps and neoplasia. Subjects should also be monitored for psychological complications related to the physical changes and deformities that can occur with the disease. As used herein, treatment can include, but does not require, resolution of the co-morbidities of acromegaly. Treatment can include, but does not require, prevention or reduction of the development of one or more of the comorbidities associated with acromegaly. As used herein, treatment for acromegaly can further include, but does not require, treatment of one or more of the comorbidities associated with acromegaly.

IX. Response Evaluation Criteria and Treatment of Cancer

Methods for detection of tumors and assessment of tumor burden are well known in the art. For example, the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines were revised in 2008 and are fully set forth in Eisenhauer *et al.*, 2009, New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur. J. Cancer. 45:228-247. These guidelines can be used to determine if a subject has tumor regression or no tumor progression as demonstrated by a complete response (CR) or partial response (PR), or stable disease (SD), respectively, as provided therein for at least a sufficient time that the CR, PR, or SD is detected meets the threshold of treatment or effective treatment as provided herein. A subject with only progressive disease (PD) after administration of an iRNA provided herein is not considered to have a favorable response to or be effectively treated by the iRNA. The development of PD after a period of CR, PR, or PD is understood as having been effectively treated by the iRNA provided herein.

It is understood that the iRNA agents provided herein can be used in conjunction with other interventions for the treatment of cancer, *e.g.*, surgery, chemotherapy, or radiation.

This invention is further illustrated by the following examples which should not be construed as limiting. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the Figure and Sequence Listing, are hereby incorporated herein by reference.

EXAMPLES

Example 1. iRNA Synthesis

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.

IGFALS Transcripts and siRNA Design

A set of siRNAs targeting the human IGFALS, "insulin-like growth factor binding protein, acid labile subunit", (human: NCBI refseqID NM_004970; NCBI GeneID: 3483), as well as toxicology-species IGFALS orthologs (cynomolgus monkey: XM_005590898; mouse: NM_008340; rat, NM_053329) were designed using custom R and Python scripts. The human NM_004970 REFSEQ mRNA has a length of 2168 bases.

The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every potential 19mer siRNA from position 50 through position 2160 (the coding region and 3' UTR) was determined with a linear model derived the direct measure of mRNA knockdown from more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. Subsets of the IGFALS siRNAs were designed with perfect or near-perfect matches between human, cynomolgus and rhesus monkey. A further subset was designed with perfect or near-perfect matches to mouse and rat IGFALS orthologs. For each strand of the siRNA, a custom Python script was used in a brute force search to measure the number and positions of mismatches between the siRNA and all potential alignments in the target species transcriptome. Extra weight was given to mismatches in the seed region, defined here as positions 2-9 of the antisense oligonucleotide, as well the cleavage site of the siRNA, defined here as positions 10-11 of the antisense oligonucleotide. The relative weight of the mismatches was 2.8; 1.2: 1 for seed mismatches, cleavage site, and other positions up through antisense position 19. Mismatches in the first position were ignored. A specificity score was calculated for each strand by summing the value of each weighted mismatch. Preference was given to siRNAs whose antisense score in human and cynomolgus monkey was >= 2.0 and predicted efficacy was >= 50% knockdown of the IGFALS transcript.

A detailed list of the unmodified IGFALS sense and antisense strand sequences is shown in Tables 3, 6, and 12.

A detailed list of the modified IGFALS sense and antisense strand sequences is shown in Tables 5, 8, and 14.

IGF-1 Transcripts and siRNA Design

A set of siRNAs targeting the human insulin like growth factor 1, "IGF1" (human: e.g., NCBI refseqID NM_000618; NCBI GeneID: 3479), as well as toxicology-species IGF1 orthologs (cynomolgus monkey: e.g., XM_005572039; mouse: e.g., NM_010512; rat, e.g., NM_178866) were designed using custom R and Python scripts. The human NM_00618 REFSEQ mRNA has a length of 7366 bases.

The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every potential 19mer siRNA from position 265 through position 7366 (the coding region and 3' UTR) was determined with a linear model derived the direct measure of mRNA knockdown from more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. Subsets of the IGF1 siRNAs were designed with perfect or near-perfect matches between human and cynomolgus monkey. A further subset was designed with perfect or near-perfect matches to human, cynomolgus monkey and mouse IGF1 orthologs. A further subset was designed with perfect or near-perfect matches to mouse and rat IGF1 orthologs. For each strand of the siRNA, a custom Python script was used in a brute force search to measure the number and positions of mismatches between the siRNA and all potential alignments in the target species transcriptome. Extra weight was given to mismatches in the seed region, defined here as positions 2-9 of the antisense oligonucleotide, as well the cleavage site of the siRNA, defined here as positions 10-11 of the antisense oligonucleotide. The relative weight of the mismatches was 2.8; 1.2: 1 for seed mismatches, cleavage site, and other positions up through antisense position 19. Mismatches in the first position were ignored. A specificity score was calculated for each strand by summing the value of each weighted mismatch. Preference was given to siRNAs whose antisense score in human and cynomolgus monkey was >= 3.0 and predicted efficacy was >= 70% knockdown of the human IGF1 transcripts.

A detailed list of the unmodified IGF-1 sense and antisense strand sequences is shown in Tables 9, 15, and 18.

A detailed list of the modified IGF-1 sense and antisense strand sequences is shown in Tables 11, 17, and 20.

siRNA Synthesis

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 are 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) is 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 is performed using TEA.3HF (triethylamine trihydro fluoride) reagent. To the methylamine deprotection solution, 200uL of dimethyl sulfoxide (DMSO) and 300ul TEA.3HF reagent were 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 is precipitated by addition of 1mL of acetontile: ethanol mixture (9:1). The plates are cooled at -80 C for 2 hours, superanatant was decanted carefully with the aid of a multi channel pipette. The oligonucleotide pellet was re-suspended in 20mM NaOAc buffer and is 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 are 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.

Example 2 - *In vitro* screening

Cell culture and transfections

Hep3B (ATCC) were transfected by adding 4.9µl 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. Firty µl of DMEM containing ~5 x10^3 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10nM and 0.1nM and in some cases 1nM 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, 50µl of Lysis/Binding Buffer and 25µl of lysis

buffer containing 3µl 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 150µl Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150µl Elution Buffer, re-captured and supernatant removed.

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

Ten µl of a master mix containing 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl 10x Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl 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 hours 37°C. Plates were then incubated at 81°C for 8 minutes.

Real time PCR

Two µl of cDNA were added to a master mix containing 0.5µl of GAPDH TaqMan Probe (Hs99999905 m1), 0.5µl IGFALS probe (HS00744047 S1) and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) 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 naïve cells or 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 10nM AD-1955, or mock transfected cells.

Table 2. Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation	**Nucleotide(s)**
A	Adenosine-3'-phosphate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gf	2'-fluoroguanosine-3'-phosphate

Abbreviation	Nucleotide(s)
Gfs	2’-fluoroguanosine-3’-phosphorothioate
Gs	guanosine-3’-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
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
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	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol Hyp-(GalNAc-alkyl)3
dT	2`-deoxythymidine-3`-phosphate
dC	2`-deoxycytidine-3`-phosphate
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofurane-5-phosphate)
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
P	Phosphate
VP	Vinyl-phosphate
(Aam)	2`-O-(N-methylacetamide)adenosine-3`-phosphate

Table 3. Unmodified Sense and Antisense Strand Sequences of IGFALS dsRNAs

Duplex name	Sense name	Sense Sequence	Position in NM 004970.2	SEQ ID NO	Antisense name	Antisense Sequence	Position in NM 004970.2	SEQ ID NO
AD-62728	A-125826	ACAGAUGAGCUCAGCGUCUUU	196-216	28	A-125827	AAAGACGCUGAGCUCAUCUGUGU	194-216	85
AD-62741	A-125832	AGCUCAGCGUCUUUUGCAGUU	203-223	29	A-125833	AACUGCAAAAGACGCUGAGCUCA	201-223	86
AD-68729	A-138233	CUGUGGCUGGACGGCAACAAA	318-337 C21A	30	A-138234	UUUGUUGCCGUCCAGCCACAGGG	316-337 C21A	87
AD-68720	A-138215	GGACGGCAACAACCUCUCGUA	326-345 C21A	31	A-138216	UACGAGAGGUUGUUGCCGUCCAG	324-345 C21A	88
AD-68717	A-138209	AACCGUCUGAGCAGGCUGGAA	549-568 G21A	32	A-138210	UUCCAGCCUGCUCAGACGGUUGU	547-568 G21A	89
AD-62737	A-125830	GGACCUCAACCUGGGUUGGAA	558-578	33	A-125831	UUCCAACCCAGGUUGAGGUCCCA	556-578	90
AD-62713	A-125820	UGGCAACAAACUGACUUACCU	645-665	34	A-125821	AGGUAAGUCAGUUUGUUGCCAGC	643-665	91
AD-62742	A-125786	GGUGCUGGCGGGCAACAGGCU	678-698	35	A-125787	AGCCUGUUGCCCGCCAGCACCAG	676-698	92
AD-62719	A-125838	GCUGGACCUGAGCAGGAACGA	747-767 C21A	36	A-125839	UCGUUCCUGCUCAGGUCCAGCUC	745-767 C21A	93
AD-62724	A-125840	CUGGACCUGAGCAGGAACGCA	748-768 G21A	37	A-125841	UGCGUUCCUGCUCAGGUCCAGCU	746-768 G21A	94
AD-68728	A-138231	GGCCAUCAAGGCAAACGUGUU	776-795	38	A-138232	AACACGUUUGCCUUGAUGGCCCG	774-795	95
AD-62717	A-125806	GCAACCUCAUCGCUGCCGUGA	833-853 G21A	39	A-125807	UCACGGCAGCGAUGAGGUUGCGG	831-853 G21A	96
AD-62731	A-125796	GCUGCCGUGGCCCCGGGCGCA	844-864 C21A	40	A-125797	UGCGCCCGGGGCCACGGCAGCGA	842-864 C21A	97
AD-62726	A-125794	UGGCCCCGGGCGCCUUCCUGA	851-871 G21A	41	A-125795	UCAGGAAGGCGCCCGGGGCCACG	849-871 G21A	98
AD-68727	A-138229	GGGCCUGAAGGCGCUGCGAUA	872-891 G21A	42	A-138230	UAUCGCAGCGCCUUCAGGCCCAG	870-891 G21A	99
AD-62715	A-125774	GCGUGGCUGGCCUCCUGGAGA	911-931 G21A	43	A-125775	UCUCCAGGAGGCCAGCCACGCGG	909-931 G21A	100
AD-68710	A-138195	GGCCUCCUGGAGGACACGUUA	921-940 C21A	44	A-138196	UAACGUGUCCUCCAGGAGGCCAG	919-940 C21A	101
AD-62743	A-125802	UGGGCCACAACCGCAUCCGGA	1043-1063 C21A	45	A-125803	UCCGGAUGCGGUUGUGGCCCAGC	1041-1063 C21A	102
AD-62711	A-125788	CCACAACCGCAUCCGGCAGCU	1047-1067	46	A-125789	AGCUGCCGGAUGCGGUUGUGGCC	1045-1067	103
AD-68709	A-138193	AGCUGGCUGAGCGCAGCUUUA	1066-1085 G21A	47	A-138194	UAAAGCUGCGCUCAGCCAGCUGC	1064-1085 G21A	104
AD-68724	A-138223	CUCACGCUAGACCACAACCAA	1110-1129 G21A	48	A-138224	UUGGUUGUGGUCUAGCGUGAGCA	1108-1129 G21A	105

Duplex name	Sense name	Sense Sequence	Position in NM 004970.2	SEQ ID NO	Antisense name	Antisense Sequence	Position in NM 004970.2	SEQ ID NO
AD-62734	A-125782	CCUCACCAACGUGGCGGUCAU	1161-1181	49	A-125783	AUGACCGCCACGUUGGUGAGGCC	1159-1181	106
AD-68111	A-135415	CCUCACCAACGUGGCGGUCAU	1161-1181	50	A-135416	AUGACCGCCACGUUGGUGAGGCC	1159-1181	107
AD-68719	A-138213	CACCAACGUGGCGGUCAUGAA	1166-1185	51	A-138214	UUCAUGACCGCCACGUUGGUGAG	1164-1185	108
AD-68712	A-138199	ACCAACGUGGCGGUCAUGAAA	1167-1186 C21A	52	A-138200	UUUCAUGACCGCCACGUUGGUGA	1165-1186 C21A	109
AD-62730	A-125780	UCAUGAACCUCUCUGGGAACU	1178-1198	53	A-125781	AGUUCCCAGAGAGGUUCAUGACC	1176-1198	110
AD-68711	A-138197	ACCUCUCUGGGAACUGUCUCA	1186-1205 C21A	54	A-138198	UGAGACAGUUCCCAGAGAGGUUC	1184-1205 C21A	111
AD-68713	A-138201	CUCUCUGGGAACUGUCUCCGA	1188-1207 G21A	55	A-138202	UCGGAGACAGUUCCCAGAGAGGU	1186-1207 G21A	112
AD-62732	A-125812	GAACUGUCUCCGGAACCUUCA	1194-1214 C21A	56	A-125813	UGAAGGUUCCGGAGACAGUUCCC	1192-1214 C21A	113
AD-68715	A-138205	GGAACUGUCUCCGGAACCUUA	1195-1214 C21A	57	A-138206	UAAGGUUCCGGAGACAGUUCCCA	1193-1214 C21A	114
AD-62738	A-125784	CUGUCUCCGGAACCUUCCGGA	1197-1217	58	A-125785	UCCGGAAGGUUCCGGAGACAGUU	1195-1217	115
AD-62736	A-125814	GUCUCCGGAACCUUCCGGAGA	1199-1219 C21A	59	A-125815	UCUCCGGAAGGUUCCGGAGACAG	1197-1219 C21A	116
AD-62712	A-125804	CGGAACCUUCCGGAGCAGGUA	1204-1224 G21A	60	A-125805	UACCUGCUCCGGAAGGUUCCGGA	1202-1224 G21A	117
AD-68723	A-138221	AACCUUCCGGAGCAGGUGUUA	1209-1228 C21A	61	A-138222	UAACACCUGCUCCGGAAGGUUCC	1207-1228 C21A	118
AD-62739	A-125800	CUUCCGGAGCAGGUGUUCCGA	1210-1230 G21A	62	A-125801	UCGGAACACCUGCUCCGGAAGGU	1208-1230 G21A	119
AD-62723	A-125824	CAUCUCCAGCAUCGAAGAACA	1302-1322	63	A-125825	UGUUCUUCGAUGCUGGAGAUGCU	1300-1322	120
AD-62745	A-125834	CUCCAGCAUCGAAGAACAGAA	1305-1325 G21A	64	A-125835	UUCUGUUCUUCGAUGCUGGAGAU	1303-1325 G21A	121
AD-62733	A-125828	CAGCAUCGAAGAACAGAGCCU	1308-1328	65	A-125829	AGGCUCUGUUCUUCGAUGCUGGA	1306-1328	122
AD-68718	A-138211	UUCCUCAAGGACAACGGCCUA	1329-1348 C21A	66	A-138212	UAGGCCGUUGUCCUUGAGGAAGA	1327-1348 C21A	123
AD-62744	A-125818	AAGGACAACGGCCUCGUGGGA	1333-1353 C21A	67	A-125819	UCCCACGAGGCCGUUGUCCUUGA	1331-1353 C21A	124
AD-68721	A-138217	GCUGCUGGAGCUCGACCUGAA	1388-1407 C21A	68	A-138218	UUCAGGUCGAGCUCCAGCAGCUC	1386-1407 C21A	125
AD-62727	A-125810	UGACCUCCAACCAGCUCACGA	1403-1423 C21A	69	A-125811	UCGUGAGCUGGUUGGAGGUCAGG	1401-1423 C21A	126
AD-62740	A-125816	CAACCAGCUCACGCACCUGCA	1410-1430 C21A	70	A-125817	UGCAGGUGCGUGAGCUGGUUGGA	1408-1430 C21A	127
AD-62716	A-125790	UGGAGUACCUGCUGCUCUCCA	1460-1480 C21A	71	A-125791	UGGAGAGCAGCAGGUACUCCAGC	1458-1480 C21A	128
AD-62725	A-125778	UGCAGCGGGCCUUCUGGCUGA	1523-1543 G21A	72	A-125779	UCAGCCAGAAGGCCCGCUGCAGG	1521-1543 G21A	129

Duplex name	Sense name	Sense Sequence	Position in NM 004970.2	SEQ ID NO	Antisense name	Antisense Sequence	Position in NM 004970.2	SEQ ID NO
AD-62714	A-125836	GCAGCGGGCCUUCUGGCUGGA	1524-1544	73	A-125837	UCCAGCCAGAAGGCCCGCUGCAG	1522-1544	130
AD-68716	A-138207	UUCUGGCUGGACGUCUCGCAA	1536-1555 C21A	74	A-138208	UUGCGAGACGUCCAGCCAGAAGG	1534-1555 C21A	131
AD-62721	A-125792	GGCUGGACGUCUCCGCACAACA	1538-1558 C21A	75	A-125793	UGUUGUGCGAGACGUCCAGCCAG	1536-1558 C21A	132
AD-62718	A-125822	UCAGGAAUAACUCCUUGCAGA	1577-1597	76	A-125823	UCUGCAAGGAGUUAUUCCUGAGG	1575-1597	133
AD-68722	A-138219	UCAGCCUCAGGAACAACUCAA	1615-1634 C21A	77	A-138220	UUGAGUUGUUCCUGAGGCUGAGG	1613-1634 C21A	134
AD-68725	A-138225	CAGCCUCAGGAACAACUCACU	1616-1635	78	A-138226	AGUGAGUUGUUCCUGAGGCUGAG	1614-1635	135
AD-68714	A-138203	AGCCUCAGGAACAACUCACUA	1617-1636 G21A	79	A-138204	UAGUGAGUUGUUCCUGAGGCUGA	1615-1636 G21A	136
AD-62722	A-125808	UCCAGGCCAUCUGUGAGGGGA	1766-1786 G21A	80	A-125809	UCCCCUCACAGAUGGCCUGGACG	1764-1786 G21A	137
AD-62735	A-125798	GGGGACAGGUCCUCAGUGUCA	1956-1976 C21A	81	A-125799	UGACACUGAGGACCUGUCCCCAG	1954-1976 C21A	138
AD-68731	A-138237	UGUCAUCAAUUAAAGGCAAAA	2054-2073 G21A	82	A-138238	UUUUGCCUUUAAUUGAUGACAGC	2052-2073 G21A	139
AD-68730	A-138235	UCAAUUAAAGGCAAAGGCAAU	2059-2078	83	A-138236	AUUGCCUUUGCCUUUAAUUGAUG	2057-2078	140
AD-68726	A-138227	AAAGGCAAAGGCAAUCGAAUA	2065-2084 C21A	84	A-138228	UAUUCGAUUGCCUUUGCCUUUAA	2063-2084 C21A	141

Table 4. IGFALS Single Dose Screen in Hep3B Cells

	Hep3B					
DuplexID	**10nM Avg**	**10nM SD**	**1nM Avg**	**1nM SD**	**0.1nM Avg**	**0.1nM SD**
AD-68729	53.9	16.0	57.5	3.8	97.4	7.0
AD-68720	48.4	4.1	73.2	27.9	116.3	9.3
AD-68717	22.6	5.6	55.4	8.3	101.8	26.9
AD-62742	126.5	20.7	ND		124.7	13.1
AD-62719	106.8	25.1	ND		66.5	4.7
AD-62724	87.8	8.4	ND		75.1	5.7
AD-68728	56.3	7.6	78.6	4.4	110.6	26.0
AD-62717	98.6	1.7	ND		102.9	56.2
AD-62731	105.7	3.4	ND		51.1	18.3
AD-62726	70.7	37.7	ND		93.5	8.9
AD-68727	68.9	14.6	94.8	12.8	124.7	23.3
AD-62715	118.5	41.8	ND		128.4	17.9
AD-68710	91.0	34.2	91.7	14.2	90.5	1.1
AD-62743	81.6	18.1	ND		123.9	20.9
AD-62711	107.0	11.3	ND		92.0	11.5
AD-68709	42.4	2.2	38.1	1.4	76.1	1.4
AD-68724	53.5	16.7	40.9	7.9	73.8	1.2
AD-62734	43.1	29.6	ND		115.2	0.3
AD-68111	29.5	14.8	37.5	0.7	92.1	3.9
AD-68719	45.4	18.9	59.6	5.7	108.7	25.0
AD-68712	40.8	1.9	58.0	4.7	97.4	13.1
AD-62730	98.4	14.2	ND		119.5	17.2
AD-68711	97.3	23.0	86.6	5.7	110.7	14.8
AD-68713	79.6	10.0	93.6	23.9	103.3	4.2
AD-62732	89.0	13.3	ND		66.0	14.3
AD-68715	48.7	13.1	71.9	6.8	78.7	7.2
AD-62738	133.2	34.5	ND		123.4	13.0
AD-62736	113.6	22.9	ND		84.9	13.9
AD-62712	68.5	22.6	ND		96.9	25.6
AD-68723	83.3	14.5	84.4	13.8	71.8	25.4
AD-62739	99.4	13.8	ND		105.6	24.4
AD-68718	83.1	6.8	78.2	0.1	119.8	36.8
AD-62744	98.8	10.4	ND		139.1	24.7
AD-68721	61.8	8.0	81.3	4.2	99.2	14.2
AD-62727	91.6	25.9	ND		86.6	46.5
AD-62740	138.9	16.0	ND		117.3	55.5
AD-62716	81.5	0.2	ND		127.8	20.9
AD-62725	109.1	6.1	ND		103.8	17.0
AD-62714	73.9	8.9	ND		64.0	16.0

	Hep3B					
DuplexID	**10nM Avg**	**10nM SD**	**1nM Avg**	**1nM SD**	**0.1nM Avg**	**0.1nM SD**
AD-68716	18.2	0.3	28.6	17.6	40.2	10.8
AD-62721	62.0	7.7	ND		91.7	6.1
AD-68722	19.2	0.5	51.1	0.7	53.4	22.2
AD-68725	20.3	7.5	23.0	6.2	67.6	15.6
AD-68714	58.9	2.8	73.5	19.0	92.6	8.9
AD-62722	120.7	69.9	ND		115.0	25.4
AD-62735	60.2	29.8	ND		100.2	22.6
AD-68731	33.5	27.2	11.8	0.9	26.6	7.4
AD-68730	14.5	0.9	24.5	7.5	44.4	8.3
AD-68726	17.0	10.0	28.6	11.8	64.5	3.7
AD-62728	46.3	20.5	ND		49.5	10.2
AD-62741	116.8	48.6	ND		143.6	35.9
AD-62737	94.7	8.6	ND		75.2	55.1
AD-62713	83.0	20.3	ND		89.3	31.9
AD-62723	103.5	32.2	ND		66.5	22.2
AD-62745	66.7	4.4	ND		85.7	61.2
AD-62733	107.1	1.3	ND		35.9	3.8
AD-62718	129.6	42.7	ND		87.7	39.2
AD-1955	102.5	25.0				
Mock	103.0	18.8				
Naïve	118.0	23.5				

Data are expressed as percent message remaining relative to AD-1955 non-targeting control.

Table 5. IGFALS Modified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	Start Position in SEQ ID NO:1
AD-68729	A-138233	csusguggCfuGfGfAfcggcaacaaaL96	142	A-138234	usUfsuguUfgCfCfguccAfgCfcacagsgsg	199	316
AD-68720	A-138215	gsgsacggCfaAfCfAfaccucucguaL96	143	A-138216	usAfscgaGfaGfGfuuguUfgCfcguccsasg	200	324
AD-68717	A-138209	asasccguCfuGfAfAfGfcaggcuggaaL96	144	A-138210	usUfsccaGfcCfUfgcucAfgAfcgguusgsu	201	547
AD-62742	A-125786	GfsgsUfgCfuGfgCfGfGfgCfaAfcAfgGfcUfL96	145	A-125787	asGfscCfuGfuUfgCfccgCfcAfgCfaCfcsasg	202	676
AD-62719	A-125838	GfscsUfgGfaCfcUfGfAfgCfaGfgAfaCfgAfL96	146	A-125839	usCfsgUfuCfcUfgCfucaGfgUfcCfaGfcsusc	203	745
AD-62724	A-125840	CfsusGfgAfcCfuGfAfGfcAfgGfaAfcGfcAfL96	147	A-125841	usGfscGfuUfcCfuGfcucAfgGfuCfcAfgscsu	204	746
AD-68728	A-138231	gsgsccauCfaAfGfGfcaaacguguuL96	148	A-138232	asAfscacGfuUfUfgccuUfgAfuggccscsg	205	774
AD-62717	A-125806	GfscsAfaCfcUfcAfUfCfgCfuGfcCfgUfgAfL96	149	A-125807	usCfsaCfgGfcAfgCfgauGfaGfgUfuGfcsgsg	206	831
AD-62731	A-125796	GfscsUfgCfcGfuGfGfCfcCfcGfgGfcGfcAfL96	150	A-125797	usGfscGfcCfcGfgGfgccAfcGfgCfaGfcsgsa	207	842
AD-62726	A-125794	UfsgsGfcCfcCfgGfGfCfgCfcUfuCfcUfgAfL96	151	A-125795	usCfsaGfgAfaGfgCfgccCfgGfgGfcCfascsg	208	849
AD-68727	A-138229	gsgsgccuGfaAfGfGfcgcugcgauaL96	152	A-138230	usAfsucgCfaGfCfgccuUfcAfggcccsasg	209	870
AD-62715	A-125774	GfscsGfuGfgCfuGfGfCfcUfcCfuGfgAfgAfL96	153	A-125775	usCfsuCfcAfgGfaGfgccAfgCfcAfcGfcsgsg	210	909
AD-68710	A-138195	gsgsccucCfuGfGfAfggacacguuaL96	154	A-138196	usAfsacgUfgUfCfcuccAfgGfaggccsasg	211	919
AD-62743	A-125802	UfsgsGfgCfcAfcAfAfCfcGfcAfuCfcGfgAfL96	155	A-125803	usCfscGfgAfuGfcGfguuGfuGfgCfcCfasgsc	212	1041
AD-62711	A-125788	CfscsAfcAfaCfcGfCfAfuCfcGfgCfaGfcUfL96	156	A-125789	asGfscUfgCfcGfgAfugcGfgUfuGfuGfgscsc	213	1045
AD-68709	A-138193	asgscuggCfuGfAfGfcgcagcuuuaL96	157	A-138194	usAfsaagCfuGfCfgcucAfgCfcagcusgsc	214	1064
AD-68724	A-138223	csuscacgCfuAfGfAfccacaaccaaL96	158	A-138224	usUfsgguUfgUfGfgucuAfgCfgugagscsa	215	1108
AD-62734	A-125782	CfscsUfcAfcCfaAfCfGfuGfgCfgGfuCfaUfL96	159	A-125783	asUfsgAfcCfgCfcAfcguUfgGfuGfaGfgscsc	216	1159
AD-68111	A-135415	cscsucacCfaAfCfGfuggcggucauL96	160	A-135416	asUfsgacCfgCfCfacguUfgGfugaggscsc	217	1159
AD-68719	A-138213	csasccaaCfgUfGfGfcggucaugaaL96	161	A-138214	usUfscauGfaCfCfgccaCfgUfuggugsasg	218	1164
AD-68712	A-138199	ascscaacGfuGfGfCfggucaugaaaL96	162	A-138200	usUfsucaUfgAfCfcgccAfcGfuuggusgsa	219	1165
AD-62730	A-125780	UfscsAfuGfaAfcCfUfCfuCfuGfgGfaAfcUfL96	163	A-125781	asGfsuUfcCfcAfgAfgagGfuUfcAfuGfascsc	220	1176
AD-68711	A-138197	ascscucuCfuGfGfGfaacugucucaL96	164	A-138198	usGfsagaCfaGfUfucccAfgAfgaggususc	221	1184
AD-68713	A-138201	csuscucuGfgGfAfAfcugucuccgaL96	165	A-138202	usCfsggaGfaCfAfguucCfcAfgagagsgsu	222	1186

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	Start Position in SEQ ID NO:1
AD-62732	A-125812	GfsasAfcUfgUfcUfCfCfgGfaAfcCfuUfcAfL96	166	A-125813	usGfsaAfgGfuUfcCfggaGfaCfaGfuUfcscsc	223	1192
AD-68715	A-138205	gsgsaacuGfuCfUfCfcggaaccuuaL96	167	A-138206	usAfsaggUfuCfCfggagAfcAfguuccscsa	224	1193
AD-62738	A-125784	CfsusGfuCfuCfcGfGfAfaCfcUfuCfcGfgAfL96	168	A-125785	usCfscGfgAfaGfgUfuccGfgAfgAfcAfgsusu	225	1195
AD-62736	A-125814	GfsusCfuCfcGfgAfAfCfcUfuCfcGfgAfgAfL96	169	A-125815	usCfsuCfcGfgAfaGfguuCfcGfgAfgAfcsasg	226	1197
AD-62712	A-125804	CfsgsGfaAfcCfuUfCfCfgGfaGfcAfgGfuAfL96	170	A-125805	usAfscCfuGfcUfcCfggaAfgGfuUfcCfgsgsa	227	1202
AD-68723	A-138221	asascccuuCfcGfGfAfgcaggugguuaL96	171	A-138222	usAfsacaCfcUfGfcuccGfgAfagguuscsc	228	1207
AD-62739	A-125800	CfsusUfcCfgGfaGfCfAfgGfuGfuUfcCfgAfL96	172	A-125801	usCfsgGfaAfcAfcCfugcUfcCfgGfaAfgsgsu	229	1208
AD-68718	A-138211	ususcccucAfaGfGfAfcaacggccuaL96	173	A-138212	usAfsggcCfgUfUfguccUfuGfaggaasgsa	230	1327
AD-62744	A-125818	AfsasGfgAfcAfaCfGfGfcCfuCfgUfgGfgAfL96	174	A-125819	usCfscCfaCfgAfgGfccgUfuGfuCfcUfusgsa	231	1331
AD-68721	A-138217	gscsugcuGfgAfGfCfucgaccugaaL96	175	A-138218	usUfscagGfuCfGfagcuCfcAfgcagcsusc	232	1386
AD-62727	A-125810	UfsgsAfcCfuCfcAfAfCfcAfgCfuCfaCfgAfL96	176	A-125811	usCfsgUfgAfgCfuGfguuGfgAfgGfuCfasgsg	233	1401
AD-62740	A-125816	CfsasAfcCfaGfcUfCfAfcGfcAfcCfuGfcAfL96	177	A-125817	usGfscAfgGfuGfcGfugaGfcUfgGfuUfgsgsa	234	1408
AD-62716	A-125790	UfsgsGfaGfuAfcCfUfGfcUfgCfuCfuCfcAfL96	178	A-125791	usGfsgAfgAfgCfaGfcagGfuAfcUfcCfasgsc	235	1458
AD-62725	A-125778	UfsgsCfaGfcGfgGfCfCfuUfcUfgGfcUfgAfL96	179	A-125779	usCfsaGfcCfaGfaAfggcCfcGfcUfgCfasgsg	236	1521
AD-62714	A-125836	GfscsAfgCfgGfgCfCfUfuCfuGfgCfuGfgAfL96	180	A-125837	usCfscAfgCfcAfgAfaggCfcCfgCfuGfcsasg	237	1522
AD-68716	A-138207	ususcuggCfuGfGfAfcgucucgcaaL96	181	A-138208	usUfsgcgAfgAfCfguccAfgCfcagaasgsg	238	1534
AD-62721	A-125792	GfsgsCfuGfgAfcGfUfCfuCfgCfaCfaAfcAfL96	182	A-125793	usGfsuUfgUfgCfgAfgacGfuCfcAfgCfcsasg	239	1536
AD-68722	A-138219	uscsagccUfcAfGfGfaacaacucaaL96	183	A-138220	usUfsgagUfuGfUfuccuGfaGfgcugasgsg	240	1613
AD-68725	A-138225	csasgccuCfaGfGfAfacaacucacuL96	184	A-138226	asGfsugaGfuUfGfuuccUfgAfggcugsasg	241	1614
AD-68714	A-138203	asgsccucAfgGfAfAfcaacucacuaL96	185	A-138204	usAfsgugAfgUfUfguucCfuGfaggcusgsa	242	1615
AD-62722	A-125808	UfscsCfaGfgCfcAfUfCfuGfuGfaGfgGfgAfL96	186	A-125809	usCfscCfcUfcAfcAfgauGfgCfcUfgGfascsg	243	1764
AD-62735	A-125798	GfsgsGfgAfcAfgGfUfCfcUfcAfgUfgUfcAfL96	187	A-125799	usGfsaCfaCfuGfaGfgacCfuGfuCfcCfcsasg	244	1954
AD-68731	A-138237	usgsucauCfaAfUfUfaaaggcaaaaL96	188	A-138238	usUfsuugCfcUfUfuaauUfgAfugacasgsc	245	2052
AD-68730	A-138235	uscsaauuAfaAfGfGfcaaaggcaauL96	189	A-138236	asUfsugcCfuUfUfgccuUfuAfauugasusg	246	2057
AD-68726	A-138227	asasaggcAfaAfGfGfcaaucgaauaL96	190	A-138228	usAfsuucGfaUfUfgccuUfuGfccuuusasa	247	2063
AD-62728	A-125826	AfscsAfgAfuGfaGfCfUfcAfgCfgUfcUfuUfL96	191	A-125827	asAfsaGfaCfgCfuGfagcUfcAfuCfuGfusgsu	248	774

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	Start Position in SEQ ID NO:1
AD-62741	A-125832	AfsgsCfuCfaGfcGfUfCfuUfuUfgCfaGfuUfL96	192	A-125833	asAfscUfgCfaAfaAfgacGfcUfgAfgCfuscsa	249	201
AD-62737	A-125830	GfsgsAfcCfuCfaAfCfCfuGfgGfuUfgGfaAfL96	193	A-125831	usUfscCfaAfcCfcAfgguUfgAfgGfuCfcscsa	250	556
AD-62713	A-125820	UfsgsGfcAfaCfaAfAfCfuGfaCfuUfaCfcUfL96	194	A-125821	asGfsgUfaAfgUfcAfguuUfgUfuGfcCfasgsc	251	643
AD-62723	A-125824	CfsasUfcUfcCfaGfCfAfuCfgAfaGfaAfcAfL96	195	A-125825	usGfsuUfcUfuCfgAfugcUfgGfaGfaUfgscsu	252	1300
AD-62745	A-125834	CfsusCfcAfgCfaUfCfGfaAfgAfaCfaGfaAfL96	196	A-125835	usUfscUfgUfuCfuUfcgaUfgCfuGfgAfgsasu	253	1301
AD-62733	A-125828	CfsasGfcAfuCfgAfAfGfaAfcAfgAfgCfcUfL96	197	A-125829	asGfsgCfuCfuGfuUfcuuCfgAfuGfcUfgsgsa	254	1306
AD-62718	A-125822	UfscsAfgGfaAfuAfAfCfuCfcUfuGfcAfgAfL96	198	A-125823	usCfsuGfcAfaGfgAfguuAfuUfcCfuGfasgsg	255	1575

Example 3 – Knockdown of IGFALs expression with an IGFALS siRNA decreases expression of IGF-1

A series of siRNAs targeting mouse IGFALS were designed and tested for the ability to knockdown expression of IGFALs mRNA in 6-8 week old C57Bl/6 female mice (n = 3 per group). A single 10 mg/kg dose of AD-62713, AD-62724, AD-62745, or AD-62728; or PBS control, was administered subcutaneously on day 1. On day 7, the mice were sacrificed to assess knockdown of IGFLALS mRNA in liver and IGFALS and IGF-1 protein in serum.

AD-62728 was found to be most effective in decreasing expression of IGFALS mRNA and protein. Specifically, at day 7, IFGALS mRNA expression in the liver was found to be about 15% of the PBS control. At day 7 after treatment with AD-62713 and AD-62745, IGFALS mRNA expression in the liver was found to be about 65% of the PBS control for both duplexes.

A decrease in serum IGFALS protein levels was found to correspond to the decrease in IGFALS mRNA in the liver. Specifically, AD-62728 decreased the serum IGFALS protein level to about 3.9 µg/ml, as compared to about 6.4 µg/ml in the PBS control. AD-62713 and AD-62745 decreased the serum IGFALS level to about 5.2 µg/ml and 4.6 µg/ml, respectively.

A decrease in serum IGF-1 was also observed in response to treatment with the duplexes. Specifically, AD-62727 decreased the serum IGF-1 protein level to about 13 ng/ml as compared to about 34 ng/ml in the PBS control. AD-62713 and AD-62745 decreased serum IGF-1 levels to about 20 ng/ml and 27 ng/ml, respectively.

Further, in a multidose study, AD-62728 was demonstrated to be effective in knockdown of expression of IGFALS mRNA in liver in an expected dose response manner. Specifically, C57Bl/6 female mice, 6-8 weeks of age (n = 3 per group) were administered either four doses of AD-62728 at 1 mg/kg or 3 mg/kg once weekly, or two doses at 3 mg/kg or 10 mg/kg every other week; or a PBS control. IGFALS mRNA knockdown was observed in the expected dose response manner.

Example 4 – *In vitro* Screening

Bioinformatics

A set of double stranded RNAi agents targeting human IGFALS (human NCBI refseq ID: NM_004970; NCBI GeneID: 3483, SEQ ID NO: 1) were designed using custom R and Python scripts. The human IGFALS REFSEQ mRNA has a length of 2168 bases.

The rationale and method for the set of agent designs is as follows: the predicted efficacy for every potential 19mer siRNA from position 10 through position 2168 was determined with a linear model derived the direct measure of mRNA knockdown from more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. The custom Python script built the set of agents by systematically selecting a siRNA every 11 bases along the target mRNA starting at position 10. At each of the positions, the neighboring agent (one position to the 5' end of the mRNA, one position to the 3' end of the mRNA) was swapped into the design set if the predicted efficacy was better than the efficacy at the exact every-11th siRNA. Low complexity agents, *i.e.,* those with Shannon Entropy measures below 1.35 were excluded from the set.

In vitro Dual-Glo® screening

Cell culture and transfections

Cos7 cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in DMEM (ATCC) supplemented with 10% FBS, before being released from the plate by trypsinization. Dual-Glo® Luciferase constructs were generated in the psiCHECK2 plasmid and contained approximately 2.0 kb (human) IGFALS sequences (SEQ ID NO: 23). Dual-luciferase plasmids were co-transfected with double stranded agents into 3000 cells using Lipofectamine RNAiMax (Invitrogen, Carlsbad CA. cat # 13778-150). For each well of a 384 well plate, 0.1 µl of Lipofectamine was added to 3 ng of plasmid vector and agent in 15 µl of Opti-MEM and allowed to complex at room temperature for 15 minutes. The mixture was then added to the cells resuspended in 35 ul of fresh complete media. Cells were incubated for 48 hours before luciferase was measured. Single dose experiments were performed at 10 nM final duplex concentration.

Dual-Glo® Luciferase assay

Forthy-eight hours after the siRNAs were transfected, Firefly (transfection control) and Renilla (fused to IGFALS target sequence in 3' UTR, SEQ ID NO: 23) luciferase were measured. First, media was removed from cells. Then Firefly luciferase activity was measured by adding 20 µl of Dual-Glo® Luciferase Reagent mixed with 20 µl of complete media to each well. The mixture was incubated at room temperature for 30 minutes before luminescense (500nm) was measured on a Spectramax (Molecular Devices) to detect the Firefly luciferase signal. Renilla luciferase activity was measured by adding 20ul of room temperature of Dual-Glo® Stop & Glo® Reagent to each well and the plates were incubated for 20 minutes before luminescence was again measured to determine the Renilla luciferase signal. The Dual-Glo® Stop & Glo® Reagent quenched the firefl y luciferase signal and sustained luminescence for the Renilla luciferase reaction. Double stranded RNAi agent activity was determined by normalizing the Renilla (IGFALS) signal to the Firefly (control) signal within each well. The magnitude of agent activity was then assessed relative to cells that were transfected with the same vector but were not treated with agent or were treated with a non-targeting double stranded RNAi agent. All transfections were done in quadruplicates.

Table 6. Unmodified Sense and Antisense Strand Sequences of IGFALS dsRNAs

Duplex Name	Sense Oligo Name	Sense oligo sequence	SEQ ID NO	Range	Antisense Oligo Name	Antisense oligo sequence	SEQ ID NO	Range in SEQ ID NO:1
AD-73764	A-147667	AGGGCAGGGGUGGCCGGCA	256	11-29	A-147668	UGCCGGCCACCCCUGCCCU	441	11-29
AD-73765	A-147669	CCGGCACAGCAGACGUACA	257	24-42	A-147670	UGUACGUCUGCUGUGCCGG	442	24-42
AD-73766	A-147671	AGACGUACCCUCCCUCGCU	258	34-52	A-147672	AGCGAGGGAGGGUACGUCU	443	34-52
AD-73767	A-147673	UCCCUCGCUGCCUGCCUGA	259	44-62	A-147674	UCAGGCAGGCAGCGAGGGA	444	44-62
AD-73768	A-147675	UGCCUGCAGCCUGCCCUGA	260	56-74	A-147676	UCAGGGCAGGCUGCAGGCA	445	56-74
AD-73769	A-147677	UGCCCUGCAUGCAGGAUGA	261	67-85	A-147678	UCAUCCUGCAUGCAGGGCA	446	67-85
AD-73770	A-147679	AGGAUGGCCCUGAGGAAAG	262	79-97	A-147680	CUUUCCUCAGGGCCAUCCU	447	79-97
AD-73771	A-147681	CUGAGGAAAGGAGGCCUGA	263	88-106	A-147682	UCAGGCCUCCUUUCCUCAG	448	88-106
AD-73772	A-147683	AGGCCUGGCCCUGGCGCUA	264	99-117	A-147684	UAGCGCCAGGGCCAGGCCU	449	99-117
AD-73773	A-147685	GCGCUGCUGCUGCUGUCCU	265	112-130	A-147686	AGGACAGCAGCAGCAGCGC	450	112-130
AD-73774	A-147687	UGCUGUCCUGGGUGGCACU	266	122-140	A-147688	AGUGCCACCCAGGACAGCA	451	122-140
AD-73775	A-147689	UGGCACUGGGCCCCCGCAA	267	134-152	A-147690	UUGCGGGGGCCCAGUGCCA	452	134-152
AD-73776	A-147691	GCCCCCGCAGCCUGGAGGA	268	143-161	A-147692	UCCUCCAGGCUGCGGGGGC	453	143-161
AD-73777	A-147693	UGGAGGGAGCAGACCCCGA	269	155-173	A-147694	UCGGGGUCUGCUCCCUCCA	454	155-173
AD-73778	A-147695	AGACCCCGGAACGCCGGGA	270	165-183	A-147696	UCCCGGCGUUCCGGGGUCU	455	165-183
AD-73779	A-147697	CGCCGGGGGAAGCCGAGGA	271	176-194	A-147698	UCCUCGGCUUCCCCCGGCG	456	176-194
AD-73780	A-147699	CGAGGGCCCAGCGUGCCCA	272	189-207	A-147700	UGGGCACGCUGGGCCCUCG	457	189-207
AD-73781	A-147701	AGCGUGCCCGGCCGCCUGU	273	198-216	A-147702	ACAGGCGGCCGGGCACGCU	458	198-216
AD-73782	A-147703	GCCUGUGUCUGCAGCUACA	274	211-229	A-147704	UGUAGCUGCAGACACAGGC	459	211-229
AD-73783	A-147705	UGCAGCUACGAUGACGACA	275	220-238	A-147706	UGUCGUCAUCGUAGCUGCA	460	220-238
AD-73784	A-147707	GACGACGCGGAUGAGCUCA	276	232-250	A-147708	UGAGCUCAUCCGCGUCGUC	461	232-250
AD-73785	A-147709	AUGAGCUCAGCGUCUUCUA	277	242-260	A-147710	UAGAAGACGCUGAGCUCAU	462	242-260
AD-73786	A-147711	UCUUCUGCAGCUCCAGGAA	278	254-272	A-147712	UUCCUGGAGCUGCAGAAGA	463	254-272
AD-73787	A-147713	UCCAGGAACCUCACGCGCA	279	265-283	A-147714	UGCGCGUGAGGUUCCUGGA	464	265-283
AD-73788	A-147715	UCACGCGCCUGCCUGAUGA	280	275-293	A-147716	UCAUCAGGCAGGCGCGUGA	465	275-293

Duplex Name	Sense Oligo Name	Sense oligo sequence	SEQ ID NO	Range	Antisense Oligo Name	Antisense oligo sequence	SEQ ID NO	Range in SEQ ID NO:1
AD-73789	A-147717	UGAUGGAGUCCCGGGCGGA	281	288-306	A-147718	UCCGCCCGGGACUCCAUCA	466	288-306
AD-73790	A-147719	CGGGCGGCACCCAAGCCCU	282	299-317	A-147720	AGGGCUUGGGUGCCGCCCG	467	299-317
AD-73791	A-147721	CAAGCCCUGUGGCUGGACA	283	310-328	A-147722	UGUCCAGCCACAGGGCUUG	468	310-328
AD-73792	A-147723	UGGCUGGACGGCAACAACA	284	319-337	A-147724	UGUUGUUGCCGUCCAGCCA	469	319-337
AD-73793	A-147725	AACAACCUCUCGUCCGUCA	285	331-349	A-147726	UGACGGACGAGAGGUUGUU	470	331-349
AD-73794	A-147727	UCCGUCCCCCCGGCAGCCU	286	343-361	A-147728	AGGCUGCCGGGGGGACGGA	471	343-361
AD-73795	A-147729	CGGCAGCCUUCCAGAACCU	287	353-371	A-147730	AGGUUCUGGAAGGCUGCCG	472	353-371
AD-73796	A-147731	CAGAACCUCUCCAGCCUGA	288	364-382	A-147732	UCAGGCUGGGAGAGGUUCUG	473	364-382
AD-73797	A-147733	AGCCUGGGCUUCCUCAACA	289	376-394	A-147734	UGUUGAGGAAGCCCCAGGCU	474	376-394
AD-73798	A-147735	UUCCUCAACCUGCAGGGCA	290	385-403	A-147736	UGCCCUGCAGGUUGAGGAA	475	385-403
AD-73799	A-147737	CAGGGCGGCCAGCUGGGCA	291	397-415	A-147738	UGCCCAGCUGGCCGCCCUG	476	397-415
AD-73800	A-147739	AGCUGGGCAGCCUGGAGCA	292	407-425	A-147740	UGCUCCAGGCUGCCCAGCU	477	407-425
AD-73801	A-147741	CUGGAGCCACAGGCGCUGA	293	418-436	A-147742	UCAGCGCCUGUGGCUCCAG	478	418-436
AD-73802	A-147743	CGCUGCUGGGCCUAGAGAA	294	431-449	A-147744	UUCUCUAGGCCCAGCAGCG	479	431-449
AD-73803	A-147745	CUAGAGAACCUGUGCCACA	295	442-460	A-147746	UGUGGCACAGGUUCUCUAG	480	442-460
AD-73804	A-147747	UGUGCCACCUGCACCUGGA	296	452-470	A-147748	UCCAGGUGCAGGUGGCACA	481	452-470
AD-73805	A-147749	ACCUGGAGCGGAACCAGCU	297	464-482	A-147750	AGCUGGUUCCGCUCCAGGU	482	464-482
AD-73806	A-147751	AACCAGCUGCGCAGCCUGA	298	475-493	A-147752	UCAGGCUGCGCAGCUGGUU	483	475-493
AD-73807	A-147753	CGCAGCCUGGCACUCGGCA	299	484-502	A-147754	UGCCGAGUGCCAGGCUGCG	484	484-502
AD-73808	A-147755	UCGGCACGUUUGCACACAA	300	497-515	A-147756	UUGUGUGCAAACGUGCCGA	485	497-515
AD-73809	A-147757	UUGCACACACGCCCGCGCU	301	506-524	A-147758	AGCGCGGGCGUGUGUGCAA	486	506-524
AD-73810	A-147759	CCCGCGCUGGCCUCGCUCA	302	517-535	A-147760	UGAGCGAGGCCAGCGCGGG	487	517-535
AD-73811	A-147761	UCGCUCGGCCUCAGCAACA	303	529-547	A-147762	UGUUGCUGAGGCCGAGCGA	488	529-547
AD-73812	A-147763	AGCAACAACCGUCUGAGCA	304	541-559	A-147764	UGCUCAGACGGUUGUUGCU	489	541-559
AD-73813	A-147767	CUGGAGGACGGGCUCUUCA	305	562-580	A-147768	UGAAGAGCCCGUCCUCCAG	490	562-580
AD-73814	A-147769	CUCUUCGAGGGCCUCGGCA	306	574-592	A-147770	UGCCGAGGCCCUCGAAGAG	491	574-592
AD-73815	A-147771	GGCCUCGGCAGCCUCUGGA	307	583-601	A-147772	UCCAGAGGCUGCCGAGGCC	492	583-601

Duplex Name	Sense Oligo Name	Sense oligo sequence	SEQ ID NO	Range	Antisense Oligo Name	Antisense oligo sequence	SEQ ID NO	Range in SEQ ID NO:1
AD-73816	A-147773	UCUGGGACCUCAACCUCGA	308	596-614	A-147774	UCGAGGUUGAGGUCCCAGA	493	596-614
AD-73817	A-147775	AACCUCGGCUGGAAUAGCA	309	607-625	A-147776	UGCUAUUCCAGCCGAGGUU	494	607-625
AD-73818	A-147777	UGGAAUAGCCUGGCGGUGA	310	616-634	A-147778	UCACCGCCAGGCUAUUCCA	495	616-634
AD-73819	A-147779	CGGUGCUCCCCGAUGCGGA	311	629-647	A-147780	UCCGCAUCGGGGAGCACCG	496	629-647
AD-73820	A-147781	GAUGCGGCGUUCCGCGGCA	312	640-658	A-147782	UGCCGCGGAACGCCGCAUC	497	640-658
AD-73821	A-147783	UUCCGCGGCCUGGGCAGCA	313	649-667	A-147784	UGCUGCCCAGGCCGCGGAA	498	649-667
AD-73822	A-147785	GCAGCCUGCGCGAGCUGGU	314	662-680	A-147786	ACCAGCUCGCGCAGGCUGC	499	662-680
AD-73823	A-147787	GAGCUGGUGCUGGCGGGCA	315	673-691	A-147788	UGCCCGCCAGCACCAGCUC	500	673-691
AD-73824	A-147789	CUGGCGGGCAACAGGCUGA	316	682-700	A-147790	UCAGCCUGUUGCCCGCCAG	501	682-700
AD-73825	A-147791	AGGCUGGCCUACCUGCAGA	317	694-712	A-147792	UCUGCAGGUAGGCCAGCCU	502	694-712
AD-73826	A-147793	ACCUGCAGCCCCGCGCUCUU	318	704-722	A-147794	AAGAGCGCGGGCUGCAGGU	503	704-722
AD-73827	A-147795	CGCUCUUCAGCGGCCUGGA	319	716-734	A-147796	UCCAGGCCGCUGAAGAGCG	504	716-734
AD-73828	A-147797	CGGCCUGGCCGAGCUCCGA	320	726-744	A-147798	UCGGAGCUCGGCCAGGCCG	505	726-744
AD-73829	A-147799	AGCUCCGGGAGCUGGACCU	321	737-755	A-147800	AGGUCCAGCUCCCGGAGCU	506	737-755
AD-73830	A-147801	CUGGACCUGAGCAGGAACA	322	748-766	A-147802	UGUUCCUGCUCAGGUCCAG	507	748-766
AD-73831	A-147803	AGGAACGCCGCUGCGGCCA	323	760-778	A-147804	UGGCCCGCAGCGCGUUCCU	508	760-778
AD-73832	A-147805	CGGGCCAUCAAGGCAAACA	324	772-790	A-147806	UGUUUGCCUUGAUGGCCCG	509	772-790
AD-73833	A-147807	AAGGCAAACGUGUUCGUGA	325	781-799	A-147808	UCACGAACACGUUUGCCUU	510	781-799
AD-73834	A-147809	UUCGUGCAGCUGCCCCGGA	326	793-811	A-147810	UCCGGGGCAGCUGCACGAA	511	793-811
AD-73835	A-147813	AGAAACUCUACCUGGACCA	327	815-833	A-147814	UGGUCCAGGUAGAGUUUCU	512	815-833
AD-73836	A-147815	CCUGGACCGCAACCUCAUA	328	825-843	A-147816	UAUGAGGUUGCGGUCCAGG	513	825-843
AD-73837	A-147817	CCUCAUCGCUGCCGUGGCA	329	837-855	A-147818	UGCCACGGCAGCGAUGAGG	514	837-855
AD-73838	A-147819	CGUGGCCCCGGCGCCUUA	330	849-867	A-147820	UAAGGCGCCCGGGGCCACG	515	849-867
AD-73839	A-147821	GGCGCCUUCCUGGGCCUGA	331	859-877	A-147822	UCAGGCCCAGGAAGGCGCC	516	859-877
AD-73840	A-147823	UGGGCCUGAAGGCGCUGCA	332	869-887	A-147824	UGCAGCGCCUUCAGGCCCA	517	869-887
AD-73841	A-147825	CGCUGCGAUGGCUGGACCU	333	881-899	A-147826	AGGUCCAGCCAUCGCAGCG	518	881-899
AD-73842	A-147827	UGGACCUGUCCCACAACCA	334	893-911	A-147828	UGGUUGUGGGACAGGUCCA	519	893-911

Duplex Name	Sense Oligo Name	Sense oligo sequence	SEQ ID NO	Range	Antisense Oligo Name	Antisense oligo sequence	SEQ ID NO	Range in SEQ ID NO:1
AD-73843	A-147829	CACAACCGCGUGGCUGGCA	335	904-922	A-147830	UGCCAGCCACGCGGUUGUG	520	904-922
AD-73844	A-147831	UGGCUGGCCUCCUGGAGGA	336	914-932	A-147832	UCCUCCAGGAGGCCAGCCA	521	914-932
AD-73845	A-147833	CCUGGAGGACACGUUCCCA	337	924-942	A-147834	UGGGAACGUGUCCUCCAGG	522	924-942
AD-73846	A-147835	UUCCCCGGUCUGCUGGGCA	338	937-955	A-147836	UGCCCAGCAGACCGGGGAA	523	937-955
AD-73847	A-147837	UGCUGGGCCUGCGUGUGCU	339	947-965	A-147838	AGCACACGCAGGCCCAGCA	524	947-965
AD-73848	A-147839	CGUGUGCUGCGGCUGUCCA	340	958-976	A-147840	UGGACAGCCGCAGCACACG	525	958-976
AD-73849	A-147841	CUGUCCCACAACGCCAUCA	341	970-988	A-147842	UGAUGGCGUUGUGGGACAG	526	970-988
AD-73850	A-147843	AACGCCAUCGCCAGCCUGA	342	979-997	A-147844	UCAGGCUGGCGAUGGCGUU	527	979-997
AD-73851	A-147845	AGCCUGCGGCCCCGCACCU	343	991-1009	A-147846	AGGUGCGGGGCCGCAGGCU	528	991-1009
AD-73852	A-147847	CGCACCUUCAAGGACCUGA	344	1003-1021	A-147848	UCAGGUCCUUGAAGGUGCG	529	1003-1021
AD-73853	A-147849	AAGGACCUGCACUUCCUGA	345	1012-1030	A-147850	UCAGGAAGUGCAGGUCCUU	530	1012-1030
AD-73854	A-147851	UUCCUGGAGGAGCUGCAGA	346	1024-1042	A-147852	UCUGCAGCUCCUCCAGGAA	531	1024-1042
AD-73855	A-147853	CUGCAGCUGGGCCACAACA	347	1036-1054	A-147854	UGUUGUGGCCCAGCUGCAG	532	1036-1054
AD-73856	A-147855	CCACAACCGCAUCCGGCAA	348	1047-1065	A-147856	UUGCCGGAUGCGGUUGUGG	533	1047-1065
AD-73857	A-147857	UCCGGCAGCUGGCUGAGCA	349	1058-1076	A-147858	UGCUCAGCCAGCUGCCGGA	534	1058-1076
AD-73858	A-147859	UGGCUGAGCGCAGCUUUGA	350	1067-1085	A-147860	UCAAAGCUGCGCUCAGCCA	535	1067-1085
AD-73859	A-147861	AGCUUUGAGGGCCUGGGGA	351	1078-1096	A-147862	UCCCCAGGCCCUCAAAGCU	536	1078-1096
AD-73860	A-147863	UGGGGCAGCUUGAGGUGCU	352	1091-1109	A-147864	AGCACCUCAAGCUGCCCCA	537	1091-1109
AD-73861	A-147865	UUGAGGUGCUCACGCUAGA	353	1100-1118	A-147866	UCUAGCGUGAGCACCUCAA	538	1100-1118
AD-73862	A-147867	ACGCUAGACCACAACCAGA	354	1111-1129	A-147868	UCUGGUUGUGGUCUAGCGU	539	1111-1129
AD-73863	A-147869	AACCAGCUCCAGGAGGUCA	355	1123-1141	A-147870	UGACCUCCUGGAGCUGGUU	540	1123-1141
AD-73864	A-147871	AGGAGGUCAAGGCGGGCGA	356	1133-1151	A-147872	UCGCCCGCCUUGACCUCCU	541	1133-1151
AD-73865	A-147873	CGGGCGCUUUCCUCGGCCU	357	1145-1163	A-147874	AGGCCGAGGAAAGCGCCCG	542	1145-1163
AD-73866	A-147875	CUCGGCCUCACCAACGUGA	358	1156-1174	A-147876	UCACGUUGGUGAGGCCGAG	543	1156-1174
AD-73867	A-147877	AACGUGGCGGUCAUGAACA	359	1168-1186	A-147878	UGUUCAUGACCGCCACGUU	544	1168-1186
AD-73868	A-147879	UCAUGAACCUCUCUGGGAA	360	1178-1196	A-147880	UUCCCAGAGAGGUUCAUGA	545	1178-1196
AD-73869	A-147881	UCUGGGAACUGUCUCCGGA	361	1189-1207	A-147882	UCCGGAGACAGUUCCCAGA	546	1189-1207

Duplex Name	Sense Oligo Name	Sense oligo sequence	SEQ ID NO	Range	Antisense Oligo Name	Antisense oligo sequence	SEQ ID NO	Range in SEQ ID NO:1
AD-73870	A-147883	UCUCCGGAACCUUCCGGAA	362	1200-1218	A-147884	UUCCGGAAGGUUCCGGAGA	547	1200-1218
AD-73871	A-147885	UUCCGGAGCAGGUGUUCCA	363	1211-1229	A-147886	UGGAACACCUGCUCCGGAA	548	1211-1229
AD-73872	A-147887	GGUGUUCCGGGGCCUGGGA	364	1221-1239	A-147888	UCCCAGGCCCCGGAACACC	549	1221-1239
AD-73873	A-147889	CUGGGCAAGCUGCACAGCA	365	1234-1252	A-147890	UGCUGUGCAGCUUGCCCAG	550	1234-1252
AD-73874	A-147891	UGCACAGCCUGCACCUGGA	366	1244-1262	A-147892	UCCAGGUGCAGGCUGUGCA	551	1244-1262
AD-73875	A-147895	CAGCUGCCUGGGACGCAUA	367	1266-1284	A-147896	UAUGCGUCCCAGGCAGCUG	552	1266-1284
AD-73876	A-147897	GACGCAUCCGCCCGCACAA	368	1277-1295	A-147898	UUGUGCGGGCGGAUGCGUC	553	1277-1295
AD-73877	A-147899	CGCACACCUUCACCGGCCU	369	1289-1307	A-147900	AGGCCGGUGAAGGUGUGCG	554	1289-1307
AD-73878	A-147901	UCACCGGCCUCUCGGGGCU	370	1298-1316	A-147902	AGCCCCGAGAGGCCGGUGA	555	1298-1316
AD-73879	A-147903	UCGGGGCUCCCGCCGACUCU	371	1309-1327	A-147904	AGAGUCGGCGGAGCCCCGA	556	1309-1327
AD-73880	A-147905	CGACUCUUCCUCAAGGACA	372	1321-1339	A-147906	UGUCCUUGAGGAAGAGUCG	557	1321-1339
AD-73881	A-147907	CAAGGACAACGGCCUCGUA	373	1332-1350	A-147908	UACGAGGCCGUUGUCCUUG	558	1332-1350
AD-73882	A-147909	GGCCUCGUGGGCAUUGAGA	374	1342-1360	A-147910	UCUCAAUGCCCACGAGGCC	559	1342-1360
AD-73883	A-147911	UUGAGGAGCAGAGCCUGUA	375	1355-1373	A-147912	UACAGGCUCUGCUCCUCAA	560	1355-1373
AD-73884	A-147913	AGAGCCUGUGGGGGCUGGA	376	1364-1382	A-147914	UCCAGCCCCCACAGGCUCU	561	1364-1382
AD-73885	A-147915	GGGCUGGCGGAGCUGCUGA	377	1375-1393	A-147916	UCAGCAGCUCCGCCAGCCC	562	1375-1393
AD-73886	A-147917	UGCUGGAGCUCGACCUGAA	378	1388-1406	A-147918	UUCAGGUCGAGCUCCAGCA	563	1388-1406
AD-73887	A-147919	GACCUGACCUCCAACCAGA	379	1399-1417	A-147920	UCUGGUUGGAGGUCAGGUC	564	1399-1417
AD-73888	A-147921	UCCAACCAGCUCACGCACA	380	1408-1426	A-147922	UGUGCGUGAGCUGGUUGGA	565	1408-1426
AD-73889	A-147923	ACGCACCUGCCCCACCGCA	381	1420-1438	A-147924	UGCGGUGGGGCAGGUGCGU	566	1420-1438
AD-73890	A-147925	CACCGCCUCUUCCAGGGCA	382	1432-1450	A-147926	UGCCCUGGAAGAGGCGGUG	567	1432-1450
AD-73891	A-147927	UCCAGGGCCUGGGCAAGCU	383	1442-1460	A-147928	AGCUUGCCCAGGCCCUGGA	568	1442-1460
AD-73892	A-147929	GCAAGCUGGAGUACCUGCU	384	1454-1472	A-147930	AGCAGGUACUCCAGCUUGC	569	1454-1472
AD-73893	A-147931	UACCUGCUGCUCUCCCGCA	385	1465-1483	A-147932	UGCGGGAGAGCAGCAGGUA	570	1465-1483
AD-73894	A-147933	CUCUCCCGCAACCGCCUGA	386	1474-1492	A-147934	UCAGGCGGUUGCGGGAGAG	571	1474-1492
AD-73895	A-147935	CCGCCUGGCAGAGCUGCCA	387	1485-1503	A-147936	UGGCAGCUCUGCCAGGCGG	572	1485-1503
AD-73896	A-147937	AGCUGCCGGCGGACGCCCU	388	1496-1514	A-147938	AGGGCGUCCGCCGGCAGCU	573	1496-1514

Duplex Name	Sense Oligo Name	Sense oligo sequence	SEQ ID NO	Range	Antisense Oligo Name	Antisense oligo sequence	SEQ ID NO	Range in SEQ ID NO:1
AD-73897	A-147939	GACGCCCUGGGCCCCCUGA	389	1507-1525	A-147940	UCAGGGGGCCCAGGGCGUC	574	1507-1525
AD-73898	A-147941	CCCCUGCAGCGGCCUUCU	390	1519-1537	A-147942	AGAAGGCCCGCUGCAGGGG	575	1519-1537
AD-73899	A-147943	GGGCCUUCUGGCUGGACGU	391	1529-1547	A-147944	ACGUCCAGCCAGAAGGCCC	576	1529-1547
AD-73900	A-147945	UGGACGUCUCGCACAACCA	392	1541-1559	A-147946	UGGUUGUGCGAGACGUCCA	577	1541-1559
AD-73901	A-147947	ACAACCGCCUGGAGGCAUU	393	1553-1571	A-147948	AAUGCCUCCAGGCGGUUGU	578	1553-1571
AD-73902	A-147949	GAGGCAUUGCCCAACAGCA	394	1564-1582	A-147950	UGCUGUUGGGCAAUGCCUC	579	1564-1582
AD-73903	A-147951	CAACAGCCUCUUGGCACCA	395	1575-1593	A-147952	UGGUGCCAAGAGGCUGUUG	580	1575-1593
AD-73904	A-147953	UUGGCACCACUGGGGCGGA	396	1585-1603	A-147954	UCCGCCCCAGUGGUGCCAA	581	1585-1603
AD-73905	A-147955	UGGGGCGGCUGCGCUACCU	397	1595-1613	A-147956	AGGUAGCGCAGCCGCCCCA	582	1595-1613
AD-73906	A-147957	CGCUACCUCAGCCUCAGGA	398	1606-1624	A-147958	UCCUGAGGCUGAGGUAGCG	583	1606-1624
AD-73907	A-147959	UCAGGAACAACUCACUGCA	399	1619-1637	A-147960	UGCAGUGAGUUGUUCCUGA	584	1619-1637
AD-73908	A-147961	CUCACUGCGGACCUUCACA	400	1629-1647	A-147962	UGUGAAGGUCCGCAGUGAG	585	1629-1647
AD-73909	A-147963	ACCUUCACGCCGCAGCCCA	401	1639-1657	A-147964	UGGGCUGCGGCGUGAAGGU	586	1639-1657
AD-73910	A-147965	CAGCCCCCGGGCCUGGAGA	402	1651-1669	A-147966	UCUCCAGGCCCCGGGGCUG	587	1651-1669
AD-73911	A-147967	GCCUGGAGCGCCUGUGGCU	403	1661-1679	A-147968	AGCCACAGGCGCUCCAGGC	588	1661-1679
AD-73912	A-147969	CUGUGGCUGGAGGGUAACA	404	1672-1690	A-147970	UGUUACCCUCCAGCCACAG	589	1672-1690
AD-73913	A-147971	GGUAACCCCUGGGACUGUA	405	1684-1702	A-147972	UACAGUCCCAGGGGUUACC	590	1684-1702
AD-73914	A-147973	GGGACUGUGGCUGCCCUCU	406	1694-1712	A-147974	AGAGGGCAGCCACAGUCCC	591	1694-1712
AD-73915	A-147975	UGCCCUCUCAAGGCGCUGA	407	1705-1723	A-147976	UCAGCGCCUUGAGAGGGCA	592	1705-1723
AD-73916	A-147977	CGCUGCGGGACUUCGCCCU	408	1718-1736	A-147978	AGGGCGAAGUCCCGCAGCG	593	1718-1736
AD-73917	A-147979	UUCGCCCUGCAGAACCCCA	409	1729-1747	A-147980	UGGGGUUCUGCAGGGCGAA	594	1729-1747
AD-73918	A-147981	CAGAACCCCAGUGCUGUGA	410	1738-1756	A-147982	UCACAGCACUGGGGUUCUG	595	1738-1756
AD-73919	A-147983	UGCUGUGCCCCGCUUCGUA	411	1749-1767	A-147984	UACGAAGCGGGGGCACAGCA	596	1749-1767
AD-73920	A-147985	CUUCGUCCAGGCCAUCUGU	412	1761-1779	A-147986	ACAGAUGGCCUGGACGAAG	597	1761-1779
AD-73921	A-147987	CAUCUGUGAGGGGGACGAU	413	1773-1791	A-147988	AUCGUCCCCCUCACAGAUG	598	1773-1791
AD-73922	A-147989	GGGGACGAUUGCCAGCCGA	414	1783-1801	A-147990	UCGGCUGGCAAUCGUCCCC	599	1783-1801
AD-73923	A-147991	CAGCCGCCCGCGUACACCU	415	1795-1813	A-147992	AGGUGUACGCGGGCGGCUG	600	1795-1813

Duplex Name	Sense Oligo Name	Sense oligo sequence	SEQ ID NO	Range	Antisense Oligo Name	Antisense oligo sequence	SEQ ID NO	Range in SEQ ID NO:1
AD-73924	A-147993	CGUACACCUACAACAACAU	416	1805-1823	A-147994	AUGUUGUUGUAGGUGUACG	601	1805-1823
AD-73925	A-147995	AACAACAUCACCUGUGCCA	417	1816-1834	A-147996	UGGCACAGGUGAUGUUGUU	602	1816-1834
AD-73926	A-147997	UGUGCCAGCCCGCCCGAGA	418	1828-1846	A-147998	UCUCGGGCGGGCUGGCACA	603	1828-1846
AD-73927	A-147999	CGCCCGAGGUCGUGGGGCU	419	1838-1856	A-148000	AGCCCCACGACCUCGGGCG	604	1838-1856
AD-73928	A-148001	CGUGGGGCUCGACCUGCGA	420	1848-1866	A-148002	UCGCAGGUCGAGCCCCACG	605	1848-1866
AD-73929	A-148003	ACCUGCGGGACCUCAGCGA	421	1859-1877	A-148004	UCGCUGAGGUCCCGCAGGU	606	1859-1877
AD-73930	A-148005	UCAGCGAGGCCCACUUUGA	422	1871-1889	A-148006	UCAAAGUGGGCCUCGCUGA	607	1871-1889
AD-73931	A-148007	ACUUUGCUCCCCUGCUGACA	423	1883-1901	A-148008	UGUCAGCAGGGAGCAAAGU	608	1883-1901
AD-73932	A-148009	CCUGCUGACCAGGUCCCCA	424	1892-1910	A-148010	UGGGGACCUGGUCAGCAGG	609	1892-1910
AD-73933	A-148011	UCCCCCGGACUCAAGCCCCA	425	1905-1923	A-148012	UGGGGCUUGAGUCCGGGGA	610	1905-1923
AD-73934	A-148013	CAAGCCCCGGGACUCAGGCA	426	1915-1933	A-148014	UGCCUGAGUCCCGGGGCUUG	611	1915-1933
AD-73935	A-148015	UCAGGCCCCCCACCUGGCUA	427	1927-1945	A-148016	UAGCCAGGUGGGGGGCCUGA	612	1927-1945
AD-73936	A-148017	ACCUGGCUCACCUUGUGCU	428	1937-1955	A-148018	AGCACAAGGUGAGCCAGGU	613	1937-1955
AD-73937	A-148019	UUGUGCUGGGGACAGGUCA	429	1949-1967	A-148020	UGACCUGUCCCCCAGCACAA	614	1949-1967
AD-73938	A-148021	GACAGGUCCUCAGUGUCCU	430	1959-1977	A-148022	AGGACACUGAGGACCUGUC	615	1959-1977
AD-73939	A-148023	CAGUGUCCUCAGGGGCCUA	431	1969-1987	A-148024	UAGGCCCCUGAGGACACUG	616	1969-1987
AD-73940	A-148025	GGGCCUGCCCAGUGCACUU	432	1981-1999	A-148026	AAGUGCACUGGGCAGGCCC	617	1981-1999
AD-73941	A-148027	UGCACUUGCUGGAAGACGA	433	1993-2011	A-148028	UCGUCUUCCAGCAAGUGCA	618	1993-2011
AD-73942	A-148029	UGGAAGACGCAAGGGCCUA	434	2002-2020	A-148030	UAGGCCCUUGCGUCUUCCA	619	2002-2020
AD-73943	A-148031	AGGGCCUGAUGGGGUGGAA	435	2013-2031	A-148032	UUCCACCCCAUCAGGCCCU	620	2013-2031
AD-73944	A-148033	GGGUGGAAGGCAUGGCGGA	436	2024-2042	A-148034	UCCGCCAUGCCUUCCACCC	621	2024-2042
AD-73945	A-148035	UGGCGGCCCCCCCAGCUGU	437	2036-2054	A-148036	ACAGCUGGGGGGGGCCGCCA	622	2036-2054
AD-73946	A-148037	CAGCUGUCAUCAAUUAAAG	438	2048-2066	A-148038	CUUUAAUUGAUGACAGCUG	623	2048-2066
AD-73947	A-148039	AAUUAAAGGCAAAGGCAAU	439	2059-2077	A-148040	AUUGCCUUUGCCUUUAAUU	624	2059-2077
AD-73948	A-148041	AAGGCAAUCGAAUCUAAAA	440	2070-2088	A-148042	UUUUAGAUUCGAUUGCCUU	625	2070-2088

Table 7. Human IGFALS Dual-Glo® in vitro 10nM screen

Duplex Name	Average 10nM	STDEV 10nM
AD-73764	46.26	12.94
AD-73765	15.98	9.39
AD-73766	27.71	1.81
AD-73767	29.96	5.64
AD-73768	53.53	15.85
AD-73769	50.94	18.08
AD-73770	35.55	11.71
AD-73771	30.07	11.32
AD-73772	33.23	3.56
AD-73773	11.46	4.14
AD-73774	58.80	12.47
AD-73775	108.20	18.60
AD-73776	51.88	20.74
AD-73777	30.64	7.39
AD-73778	81.00	19.34
AD-73779	78.23	16.91
AD-73780	67.63	20.32
AD-73781	75.04	41.97
AD-73782	11.25	3.14
AD-73783	84.25	27.48
AD-73784	31.16	3.50
AD-73785	40.36	15.91
AD-73786	26.61	4.91
AD-73787	37.73	13.41
AD-73788	41.39	9.64
AD-73789	69.70	17.02
AD-73790	54.70	18.10
AD-73791	37.77	14.31
AD-73792	59.22	4.58
AD-73793	30.72	11.33
AD-73794	96.09	23.63
AD-73795	27.15	4.14
AD-73796	44.57	8.83
AD-73797	22.69	5.07
AD-73798	52.76	11.72
AD-73799	69.71	10.21
AD-73800	49.18	17.49
AD-73801	59.80	17.00
AD-73802	28.96	1.45
AD-73803	33.13	19.76
AD-73804	40.68	7.80
AD-73805	63.69	6.82

Duplex Name	Average 10nM	STDEV 10nM
AD-73806	66.25	14.80
AD-73807	48.62	17.85
AD-73808	25.07	4.32
AD-73809	68.40	17.86
AD-73810	83.96	14.19
AD-73811	64.13	17.42
AD-73812	46.66	9.77
AD-73813	44.50	17.35
AD-73814	63.89	24.44
AD-73815	52.18	19.16
AD-73816	46.10	24.18
AD-73817	47.24	12.69
AD-73818	26.52	4.62
AD-73819	48.75	11.37
AD-73820	60.19	5.23
AD-73821	94.35	26.80
AD-73822	84.38	36.20
AD-73823	40.82	16.47
AD-73824	73.14	20.30
AD-73825	28.56	4.59
AD-73826	46.85	5.02
AD-73827	47.58	13.90
AD-73828	63.46	15.46
AD-73829	95.35	32.53
AD-73830	58.41	9.47
AD-73831	76.16	9.56
AD-73832	66.65	24.27
AD-73833	48.53	16.86
AD-73834	61.65	17.68
AD-73835	58.15	28.49
AD-73836	26.15	4.79
AD-73837	43.30	9.38
AD-73838	74.76	20.65
AD-73839	78.85	7.72
AD-73840	43.78	12.13
AD-73841	40.30	13.20
AD-73842	43.45	1.12
AD-73843	47.08	8.45
AD-73844	110.22	43.07
AD-73845	53.10	20.78
AD-73846	100.03	52.61
AD-73847	59.82	19.09
AD-73848	26.03	3.83
AD-73849	38.45	7.00

Duplex Name	Average 10nM	STDEV 10nM
AD-73850	86.08	20.23
AD-73851	61.41	7.67
AD-73852	53.33	19.36
AD-73853	85.67	29.83
AD-73854	54.76	5.66
AD-73855	104.89	36.39
AD-73856	57.24	13.36
AD-73857	63.18	12.14
AD-73858	20.59	3.73
AD-73859	42.26	7.68
AD-73860	94.01	20.91
AD-73861	45.90	18.39
AD-73862	26.77	5.70
AD-73863	39.07	19.21
AD-73864	59.26	14.59
AD-73865	41.82	10.07
AD-73866	60.91	19.05
AD-73867	35.80	9.83
AD-73868	46.58	6.40
AD-73869	64.22	11.51
AD-73870	80.14	7.20
AD-73871	60.16	20.80
AD-73872	56.05	24.26
AD-73873	68.99	18.51
AD-73874	110.04	18.69
AD-73875	45.34	19.36
AD-73876	51.41	17.32
AD-73877	48.52	10.40
AD-73878	114.98	63.70
AD-73879	60.09	8.24
AD-73880	38.19	8.87
AD-73881	74.45	6.60
AD-73882	33.01	9.79
AD-73883	34.58	16.31
AD-73884	53.88	4.17
AD-73885	40.86	12.23
AD-73886	48.81	15.26
AD-73887	100.05	43.02
AD-73888	52.76	9.03
AD-73889	104.07	24.09
AD-73890	34.25	10.25
AD-73891	59.05	17.53
AD-73892	43.11	18.36
AD-73893	74.85	51.34

Duplex Name	Average 10nM	STDEV 10nM
AD-73894	71.46	42.74
AD-73895	67.51	15.16
AD-73896	65.38	19.16
AD-73897	113.90	19.73
AD-73898	30.88	11.29
AD-73899	71.21	20.59
AD-73900	45.87	8.22
AD-73901	81.14	27.00
AD-73902	57.98	26.64
AD-73903	60.87	50.48
AD-73904	144.84	56.92
AD-73905	80.06	7.93
AD-73906	25.22	6.98
AD-73907	33.52	8.04
AD-73908	88.78	21.09
AD-73909	94.23	19.36
AD-73910	106.31	18.12
AD-73911	64.23	4.10
AD-73912	25.25	5.85
AD-73913	42.38	3.07
AD-73914	38.34	6.64
AD-73915	61.19	28.72
AD-73916	71.86	28.39
AD-73917	95.24	18.35
AD-73918	80.25	27.23
AD-73919	48.91	6.14
AD-73920	39.40	11.01
AD-73921	57.14	12.93
AD-73922	45.90	21.00
AD-73923	56.04	18.98
AD-73924	28.94	7.49
AD-73925	58.43	28.38
AD-73926	102.32	34.13
AD-73927	100.65	27.38
AD-73928	85.51	11.58
AD-73929	51.54	4.93
AD-73930	27.83	6.80
AD-73931	36.71	9.74
AD-73932	37.09	6.54
AD-73933	54.60	14.50
AD-73934	188.17	65.46
AD-73935	77.02	12.48
AD-73936	71.96	24.59
AD-73937	48.37	18.42

Duplex Name	Average 10nM	STDEV 10nM
AD-73938	47.06	6.65
AD-73939	55.62	19.17
AD-73940	74.83	6.45
AD-73941	41.91	18.36
AD-73942	87.02	43.38
AD-73943	47.56	6.76
AD-73944	39.62	7.36
AD-73945	61.45	10.10
AD-73946	16.22	4.18
AD-73947	17.27	8.22
AD-73948	33.62	7.51

Table 8. Modified Sense and Antisense Strand Sequences of IGFALS dsRNAs

Duplex Name	Sense Oligo Name	Sense Oligo Sequence	SEQ ID	Antisense Oligo Name	Antisense Oligo Seq	SEQ ID	mRNA target sequence	SEQ ID
AD-73764	A-147667	AGGGCAGGGGUGGCCGGCAdTdT	626	A-147668	UGCCGGCCACCCCUGCCCUdTdT	811	AGGGCAGGGGUGGCCGGCA	996
AD-73765	A-147669	CCGGCACAGCAGACGUACAdTdT	627	A-147670	UGUACGUCUGCUGUGCCGGdTdT	812	CCGGCACAGCAGACGUACC	997
AD-73766	A-147671	AGACGUACCCUCCCUCGCUdTdT	628	A-147672	AGCGAGGGAGGGUACGUCUdTdT	813	AGACGUACCCUCCCUCGCU	998
AD-73767	A-147673	UCCCUCGCUGCCUGCCUGAdTdT	629	A-147674	UCAGGCAGGCAGCGAGGGAdTdT	814	UCCCUCGCUGCCUGCCUGC	999
AD-73768	A-147675	UGCCUGCAGCCUGCCCUGAdTdT	630	A-147676	UCAGGGCAGGCUGCAGGCAdTdT	815	UGCCUGCAGCCUGCCCUGC	1000
AD-73769	A-147677	UGCCCUGCAUGCAGGAUGAdTdT	631	A-147678	UCAUCCUGCAUGCAGGGCAdTdT	816	UGCCCUGCAUGCAGGAUGG	1001
AD-73770	A-147679	AGGAUGGCCCUGAGGAAAGdTdT	632	A-147680	CUUUCCUCAGGGCCAUCCUdTdT	817	AGGAUGGCCCUGAGGAAAG	1002
AD-73771	A-147681	CUGAGGAAAGGAGGCCUGAdTdT	633	A-147682	UCAGGCCUCCUUUCCUCAGdTdT	818	CUGAGGAAAGGAGGCCUGG	1003
AD-73772	A-147683	AGGCCUGGCCCUGGCGCUAdTdT	634	A-147684	UAGCGCCAGGGCCAGGCCUdTdT	819	AGGCCUGGCCCUGGCGCUG	1004
AD-73773	A-147685	GCGCUGCUGCUGCUGUCCUdTdT	635	A-147686	AGGACAGCAGCAGCAGCGCdTdT	820	GCGCUGCUGCUGCUGUCCU	1005
AD-73774	A-147687	UGCUGUCCUGGGUGGCACUdTdT	636	A-147688	AGUGCCACCCAGGACAGCAdTdT	821	UGCUGUCCUGGGUGGCACU	1006
AD-73775	A-147689	UGGCACUGGGCCCCCGCAAdTdT	637	A-147690	UUGCGGGGGCCCAGUGCCAdTdT	822	UGGCACUGGGCCCCCGCAG	1007
AD-73776	A-147691	GCCCCCGCAGCCUGGAGGAdTdT	638	A-147692	UCCUCCAGGCUGCGGGGGCdTdT	823	GCCCCCGCAGCCUGGAGGG	1008
AD-73777	A-147693	UGGAGGGAGCAGACCCCGAdTdT	639	A-147694	UCGGGGUCUGCUCCCUCCAdTdT	824	UGGAGGGAGCAGACCCCGG	1009
AD-73778	A-147695	AGACCCCGGAACGCCGGGAdTdT	640	A-147696	UCCCGGCGUUCCGGGGUCUdTdT	825	AGACCCCGGAACGCCGGGG	1010
AD-73779	A-147697	CGCCGGGGGAAGCCGAGGAdTdT	641	A-147698	UCCUCGGCUUCCCCCGGCGdTdT	826	CGCCGGGGGAAGCCGAGGG	1011
AD-73780	A-147699	CGAGGGCCCAGCGUGCCCAdTdT	642	A-147700	UGGGCACGCUGGGCCCUCGdTdT	827	CGAGGGCCCAGCGUGCCCG	1012
AD-73781	A-147701	AGCGUGCCCGGCCGCCUGUdTdT	643	A-147702	ACAGGCGGCCGGGCACGCUdTdT	828	AGCGUGCCCGGCCGCCUGU	1013
AD-73782	A-147703	GCCUGUGUCUGCAGCUACAdTdT	644	A-147704	UGUAGCUGCAGACACAGGCdTdT	829	GCCUGUGUCUGCAGCUACG	1014
AD-73783	A-147705	UGCAGCUACGAUGACGACAdTdT	645	A-147706	UGUCGUCAUCGUAGCUGCAdTdT	830	UGCAGCUACGAUGACGACG	1015
AD-73784	A-147707	GACGACGCGGAUGAGCUCAdTdT	646	A-147708	UGAGCUCAUCCGCGUCGUCdTdT	831	GACGACGCGGAUGAGCUCA	1016
AD-73785	A-147709	AUGAGCUCAGCGUCUUCUAdTdT	647	A-147710	UAGAAGACGCUGAGCUCAUdTdT	832	AUGAGCUCAGCGUCUUCUG	1017
AD-73786	A-147711	UCUUCUGCAGCUCCAGGAAdTdT	648	A-147712	UUCCUGGAGCUGCAGAAGAdTdT	833	UCUUCUGCAGCUCCAGGAA	1018
AD-73787	A-147713	UCCAGGAACCUCACGCGCAdTdT	649	A-147714	UGCGCGUGAGGUUCCUGGAdTdT	834	UCCAGGAACCUCACGCGCC	1019
AD-73788	A-147715	UCACGCGCCUGCCUGAUGAdTdT	650	A-147716	UCAUCAGGCAGGCGCGUGAdTdT	835	UCACGCGCCUGCCUGAUGG	1020

Duplex Name	Sense Oligo Name	Sense Oligo Sequence	SEQ ID	Antisense Oligo Name	Antisense Oligo Seq	SEQ ID	mRNA target sequence	SEQ ID
AD-73789	A-147717	UGAUGGAGUCCCGGGCGGAdTdT	651	A-147718	UCCGCCCGGGACUCCAUCAdTdT	836	UGAUGGAGUCCCGGGCGGC	1021
AD-73790	A-147719	CGGGCGGCACCCAAGCCCUdTdT	652	A-147720	AGGGCUUGGGUGCCGCCCGdTdT	837	CGGGCGGCACCCAAGCCCU	1022
AD-73791	A-147721	CAAGCCCUGUGGGCUGGACAdTdT	653	A-147722	UGUCCAGCCACAGGGCUUGdTdT	838	CAAGCCCUGUGGGCUGGACG	1023
AD-73792	A-147723	UGGCUGGACGGCAACAACAdTdT	654	A-147724	UGUUGUUGCCGUCCAGCCAdTdT	839	UGGCUGGACGGCAACAACC	1024
AD-73793	A-147725	AACAACCUCUCGUCCGUCAdTdT	655	A-147726	UGACGGACGAGAGGUUGUUdTdT	840	AACAACCUCUCGUCCGUCC	1025
AD-73794	A-147727	UCCGUCCCCCCCGGCAGCCUdTdT	656	A-147728	AGGCUGCCGGGGGGACGGAdTdT	841	UCCGUCCCCCCCGGCAGCCU	1026
AD-73795	A-147729	CGGCAGCCUUCCCAGAACCUdTdT	657	A-147730	AGGUUCUGGAAGGCUGCCGdTdT	842	CGGCAGCCUUCCAGAACCU	1027
AD-73796	A-147731	CAGAACCUCUCCCAGCCUGAdTdT	658	A-147732	UCAGGCUGGAGAGGUUCUGdTdT	843	CAGAACCUCUCCCAGCCUGG	1028
AD-73797	A-147733	AGCCUGGGCUUCCUCAACAdTdT	659	A-147734	UGUUGAGGAAGCCCAGGCUdTdT	844	AGCCUGGGCUUCCUCAACC	1029
AD-73798	A-147735	UUCCUCAACCUGCAGGGCAdTdT	660	A-147736	UGCCCUGCAGGUUGAGGAAdTdT	845	UUCCUCAACCUGCAGGGCG	1030
AD-73799	A-147737	CAGGGCGGCCAGCUGGGCAdTdT	661	A-147738	UGCCCAGCUGGCCGCCCUGdTdT	846	CAGGGCGGCCAGCUGGGCA	1031
AD-73800	A-147739	AGCUGGGCAGCCCUGGAGCAdTdT	662	A-147740	UGCUCCAGGCUGCCCAGCUdTdT	847	AGCUGGGCAGCCUGGAGCC	1032
AD-73801	A-147741	CUGGAGCCCACAGGCGCUGAdTdT	663	A-147742	UCAGCGCCUGUGGGCUCCAGdTdT	848	CUGGAGCCCACAGGCGCUGC	1033
AD-73802	A-147743	CGCUGCUGGGCCUAGAGAAdTdT	664	A-147744	UUCUCUAGGCCCAGCAGCGdTdT	849	CGCUGCUGGGCCUAGAGAA	1034
AD-73803	A-147745	CUAGAGAACCUGUGCCACAdTdT	665	A-147746	UGUGGCACAGGUUCUCUAGdTdT	850	CUAGAGAACCUGUGCCACC	1035
AD-73804	A-147747	UGUGCCACCUGCACCUGGAdTdT	666	A-147748	UCCAGGUGCAGGUGGCACAdTdT	851	UGUGCCACCUGCACCUGGA	1036
AD-73805	A-147749	ACCUGGAGCGGAACCAGCUdTdT	667	A-147750	AGCUGGUUCCGCUCCAGGUdTdT	852	ACCUGGAGCGGAACCAGCU	1037
AD-73806	A-147751	AACCAGCUGCGCAGCCUGAdTdT	668	A-147752	UCAGGCUGCGCAGCUGGUUdTdT	853	AACCAGCUGCGCAGCCUGG	1038
AD-73807	A-147753	CGCAGCCUGGCACUCGGCAdTdT	669	A-147754	UGCCGAGUGCCAGGCUGCGdTdT	854	CGCAGCCUGGCACUCGGCA	1039
AD-73808	A-147755	UCGGCACGUUUGCACACAAdTdT	670	A-147756	UUGUGUGCAAACGUGCCGAdTdT	855	UCGGCACGUUUGCACACAC	1040
AD-73809	A-147757	UUGCACACACGCCCGCGCUdTdT	671	A-147758	AGCGCGGGCGUGUGUGCAAdTdT	856	UUGCACACACGCCCGCGCU	1041
AD-73810	A-147759	CCCGCGCUGGCCUCGCUCAdTdT	672	A-147760	UGAGCGAGGCCAGCGCGGGdTdT	857	CCCGCGCUGGCCUCGCUCG	1042
AD-73811	A-147761	UCGCUCGGCCUCAGCAACAdTdT	673	A-147762	UGUUGCUGAGGCCGAGCGAdTdT	858	UCGCUCGGCCUCAGCAACA	1043
AD-73812	A-147763	AGCAACAACCGUCUGAGCAdTdT	674	A-147764	UGCUCAGACGGUUGUUGCUdTdT	859	AGCAACAACCGUCUGAGCA	1044
AD-73813	A-147767	CUGGAGGACGGGCUCUUCAdTdT	675	A-147768	UGAAGAGCCCGUCCUCCAGdTdT	860	CUGGAGGACGGGCUCUUCG	1045
AD-73814	A-147769	CUCUUCGAGGGCCUCGGCAdTdT	676	A-147770	UGCCGAGGCCCUCGAAGAGdTdT	861	CUCUUCGAGGGCCUCGGCA	1046
AD-73815	A-147771	GGCCUCGGCAGCCUCUGGAdTdT	677	A-147772	UCCAGAGGCUGCCGAGGCCdTdT	862	GGCCUCGGCAGCCUCUGGG	1047

Duplex Name	Sense Oligo Name	Sense Oligo Sequence	SEQ ID	Antisense Oligo Name	Antisense Oligo Seq	SEQ ID	mRNA target sequence	SEQ ID
AD-73816	A-147773	UCUGGGACCUCAACCUCGAdTdT	678	A-147774	UCGAGGUUGAGGUCCCAGAdTdT	863	UCUGGGACCUCAACCUCGG	1048
AD-73817	A-147775	AACCUCGGCUGGAAUAGCAdTdT	679	A-147776	UGCUAUUCCAGCCGAGGUUdTdT	864	AACCUCGGCUGGAAUAGCC	1049
AD-73818	A-147777	UGGAAUAGCCUGGCGGUGAdTdT	680	A-147778	UCACCGCCAGGCUAUUCCAdTdT	865	UGGAAUAGCCUGGCGGUGC	1050
AD-73819	A-147779	CGGUGCUCCCCGAUGCGGAdTdT	681	A-147780	UCCGCAUCGGGGAGCACCGdTdT	866	CGGUGCUCCCCGAUGCGGC	1051
AD-73820	A-147781	GAUGCGGCGUUCCGCGGCAdTdT	682	A-147782	UGCCGCGGAACGCCGCAUCdTdT	867	GAUGCGGCGUUCCGCGGCC	1052
AD-73821	A-147783	UUCCGCGGCCUGGCAGCAdTdT	683	A-147784	UGCUGCCCAGGCCGCGGAAdTdT	868	UUCCGCGGCCUGGGCAGCC	1053
AD-73822	A-147785	GCAGCCUGCGCGAGCUGUdTdT	684	A-147786	ACCAGCUCGCGCAGGCUGCdTdT	869	GCAGCCUGCGCGAGCUGGU	1054
AD-73823	A-147787	GAGCUGGUGCUGGCGGGCAdTdT	685	A-147788	UGCCCGCCAGCACCAGCUCdTdT	870	GAGCUGGUGCUGGCGGGCA	1055
AD-73824	A-147789	CUGGCGGGCAACAGGCUGAdTdT	686	A-147790	UCAGCCUGUUGCCCGCCAGdTdT	871	CUGGCGGGCAACAGGCUGG	1056
AD-73825	A-147791	AGGCUGGCCUACCUGCAGAdTdT	687	A-147792	UCUGCAGGUAGGCCAGGCCUdTdT	872	AGGCUGGCCUACCUGCAGC	1057
AD-73826	A-147793	ACCUGCAGCCCGCGCUCUUdTdT	688	A-147794	AAGAGCGCGGGCUGCAGGUdTdT	873	ACCUGCAGCCCGCGCUCUU	1058
AD-73827	A-147795	CGCUCUUCAGCGGCCUGGAdTdT	689	A-147796	UCCAGGCCGCUGAAGAGCGdTdT	874	CGCUCUUCAGCGGCCUGGC	1059
AD-73828	A-147797	CGGCCUGGCCGAGCUCCGAdTdT	690	A-147798	UCGGAGCUCGGCCAGGCCGdTdT	875	CGGCCUGGCCGAGCUCCGG	1060
AD-73829	A-147799	AGCUCCGGGAGCUGGACCUdTdT	691	A-147800	AGGUCCAGCUCCCCGGAGCUdTdT	876	AGCUCCGGGAGCUGGACCU	1061
AD-73830	A-147801	CUGGACCUGAGCAGGAACAdTdT	692	A-147802	UGUUCCUGCUCAGGUCCAGdTdT	877	CUGGACCUGAGCAGGAACG	1062
AD-73831	A-147803	AGGAACGCGCUGCGGGCCAdTdT	693	A-147804	UGGCCCGCAGCGCGUUCCUdTdT	878	AGGAACGCGCUGCGGGCCA	1063
AD-73832	A-147805	CGGGCCAUCAAGGCAAACAdTdT	694	A-147806	UGUUUGCCUUGAUGGCCCGdTdT	879	CGGGCCAUCAAGGCAAACG	1064
AD-73833	A-147807	AAGGCAAACGUGUUCGUGAdTdT	695	A-147808	UCACGAACACGUUUGCCUUdTdT	880	AAGGCAAACGUGUUCGUGC	1065
AD-73834	A-147809	UUCGUGCAGCUGCCCCGGAdTdT	696	A-147810	UCCGGGGCAGCUGCACGAAdTdT	881	UUCGUGCAGCUGCCCCGGC	1066
AD-73835	A-147813	AGAAACUCUACCUGGACCAdTdT	697	A-147814	UGGUCCAGGUAGAGUUUCUdTdT	882	AGAAACUCUACCUGGACCG	1067
AD-73836	A-147815	CCUGGACCGCAACCUCAUAdTdT	698	A-147816	UAUGAGGUUGCGGUCCAGGdTdT	883	CCUGGACCGCAACCUCAUC	1068
AD-73837	A-147817	CCUCAUCGCUGCCGUGGCAdTdT	699	A-147818	UGCCACGGCAGCGAUGAGGdTdT	884	CCUCAUCGCUGCCGUGGCC	1069
AD-73838	A-147819	CGUGGCCCCGGGCGCCUUAdTdT	700	A-147820	UAAGGCGCCCGGGGCCACGdTdT	885	CGUGGCCCCGGGCGCCUUC	1070
AD-73839	A-147821	GGCGCCUUCCUGGGCCUGAdTdT	701	A-147822	UCAGGCCCAGGAAGGCGCCdTdT	886	GGCGCCUUCCUGGGCCUGA	1071
AD-73840	A-147823	UGGGCCUGAAGGCGCUGCAdTdT	702	A-147824	UGCAGCGCCUUCAGGCCCAdTdT	887	UGGGCCUGAAGGCGCUGCG	1072
AD-73841	A-147825	CGCUGCGAUGGCUGGACCUdTdT	703	A-147826	AGGUCCAGCCAUCGCAGCGdTdT	888	CGCUGCGAUGGCUGGACCU	1073
AD-73842	A-147827	UGGACCUGUCCCCACAACCAdTdT	704	A-147828	UGGUUGUGGGACAGGUCCAdTdT	889	UGGACCUGUCCCCACAACCG	1074

Duplex Name	Sense Oligo Name	Sense Oligo Sequence	SEQ ID	Antisense Oligo Name	Antisense Oligo Seq	SEQ ID	mRNA target sequence	SEQ ID
AD-73843	A-147829	CACAACCGCGUGGCUGGCAdTdT	705	A-147830	UGCCAGCCACGCGGUUGUGdTdT	890	CACAACCGCGUGGCUGGCC	1075
AD-73844	A-147831	UGGCUGGCCUCCUGGAGGAdTdT	706	A-147832	UCCUCCAGGAGGCCAGCCAdTdT	891	UGGCUGGCCUCCUGGAGGA	1076
AD-73845	A-147833	CCUGGAGGACACGUUCCCAdTdT	707	A-147834	UGGGAACGUGUCCUCCAGGdTdT	892	CCUGGAGGACACGUUCCCC	1077
AD-73846	A-147835	UUCCCCGGUCUGCUGGGCAdTdT	708	A-147836	UGCCCAGCAGACCGGGGAAdTdT	893	UUCCCCGGUCUGCUGGGCC	1078
AD-73847	A-147837	UGCUGGGCCUGCGUGUGCUdTdT	709	A-147838	AGCACACGCAGGCCCAGCAdTdT	894	UGCUGGGCCUGCGUGUGCU	1079
AD-73848	A-147839	CGUGUGCUGCGGCUGUCCAdTdT	710	A-147840	UGGACAGCCGCAGCACACGdTdT	895	CGUGUGCUGCGGCUGUCCC	1080
AD-73849	A-147841	CUGUCCCACAACGCCAUCAdTdT	711	A-147842	UGAUGGCGUUGUGGGACAGdTdT	896	CUGUCCCACAACGCCAUCG	1081
AD-73850	A-147843	AACGCCAUCGCCAGCCUGAdTdT	712	A-147844	UCAGGCUGGCGAUGGCGUUdTdT	897	AACGCCAUCGCCAGCCUGC	1082
AD-73851	A-147845	AGCCUGCGGCCCCGCACCUdTdT	713	A-147846	AGGUGCGGGGCCGCAGGCUdTdT	898	AGCCUGCGGCCCCGCACCU	1083
AD-73852	A-147847	CGCACCUUCAAGGACCUGAdTdT	714	A-147848	UCAGGUCCUUGAAGGUGCGdTdT	899	CGCACCUUCAAGGACCUGC	1084
AD-73853	A-147849	AAGGACCUGCACUUCCUGAdTdT	715	A-147850	UCAGGAAGUGCAGGUCCUUdTdT	900	AAGGACCUGCACUUCCUGG	1085
AD-73854	A-147851	UUCCUGGAGGAGCUGCAGAdTdT	716	A-147852	UCUGCAGCUCCUCCAGGAAdTdT	901	UUCCUGGAGGAGCUGCAGC	1086
AD-73855	A-147853	CUGCAGCUGGGCCACAACAdTdT	717	A-147854	UGUUGUGGCCCAGCUGCAGdTdT	902	CUGCAGCUGGGCCACAACC	1087
AD-73856	A-147855	CCACAACCGCAUCCGGCAAdTdT	718	A-147856	UUGCCGGAUGCGGUUGUGGdTdT	903	CCACAACCGCAUCCGGCAG	1088
AD-73857	A-147857	UCCGGCAGCUGGCUGAGCAdTdT	719	A-147858	UGCUCAGCCAGCUGCCGGAdTdT	904	UCCGGCAGCUGGCUGAGCG	1089
AD-73858	A-147859	UGGCUGAGCGCAGCUUUGAdTdT	720	A-147860	UCAAAGCUGCGCUCAGCCAdTdT	905	UGGCUGAGCGCAGCUUUGA	1090
AD-73859	A-147861	AGCUUUGAGGGCCUGGGGAdTdT	721	A-147862	UCCCCAGGCCCUCAAAGCUdTdT	906	AGCUUUGAGGGCCUGGGGC	1091
AD-73860	A-147863	UGGGGCAGCUUGAGGUGCUdTdT	722	A-147864	AGCACCUCAAGCUGCCCCAdTdT	907	UGGGGCAGCUUGAGGUGCU	1092
AD-73861	A-147865	UUGAGGUGCUCACGCUAGAdTdT	723	A-147866	UCUAGCGUGAGCACCUCAAdTdT	908	UUGAGGUGCUCACGCUAGA	1093
AD-73862	A-147867	ACGCUAGACCACAACCAGAdTdT	724	A-147868	UCUGGUUGUGGUCUAGCGUdTdT	909	ACGCUAGACCACAACCAGC	1094
AD-73863	A-147869	AACCAGCUCCAGGAGGUCAdTdT	725	A-147870	UGACCUCCUGGAGCUGGUUdTdT	910	AACCAGCUCCAGGAGGUCA	1095
AD-73864	A-147871	AGGAGGUCAAGGCGGGCGAdTdT	726	A-147872	UCGCCCGCCUUGACCUCCUdTdT	911	AGGAGGUCAAGGCGGGCGC	1096
AD-73865	A-147873	CGGGCGCUUUCCUCGGCCUdTdT	727	A-147874	AGGCCGAGGAAAGCGCCCGdTdT	912	CGGGCGCUUUCCUCGGCCU	1097
AD-73866	A-147875	CUCGGCCUCACCAACGUGAdTdT	728	A-147876	UCACGUUGGUGAGGCCGAGdTdT	913	CUCGGCCUCACCAACGUGG	1098
AD-73867	A-147877	AACGUGGCGGUCAUGAACAdTdT	729	A-147878	UGUUCAUGACCGCCACGUUdTdT	914	AACGUGGCGGUCAUGAACC	1099
AD-73868	A-147879	UCAUGAACCUCUCUGGGAAdTdT	730	A-147880	UUCCCAGAGAGGUUCAUGAdTdT	915	UCAUGAACCUCUCUGGGAA	1100
AD-73869	A-147881	UCUGGGAACUGUCUCCGGAdTdT	731	A-147882	UCCGGAGACAGUUCCCAGAdTdT	916	UCUGGGAACUGUCUCCGGA	1101

Duplex Name	Sense Oligo Name	Sense Oligo Sequence	SEQ ID	Antisense Oligo Name	Antisense Oligo Seq	SEQ ID	mRNA target sequence	SEQ ID
AD-73870	A-147883	UCUCCGGAACCUUCCGGAAdTdT	732	A-147884	UUCCGGAAGGUUCCGGAGAdTdT	917	UCUCCGGAACCUUCCGGAG	1102
AD-73871	A-147885	UUCCGGAGCAGGUGUUCCAdTdT	733	A-147886	UGGAACACCUGCUCCGGAAdTdT	918	UUCCGGAGCAGGUGUUCCG	1103
AD-73872	A-147887	GGUGUUCCGGGGCCUGGGAdTdT	734	A-147888	UCCCAGGCCCCGGAACACCdTdT	919	GGUGUUCCGGGGCCUGGGC	1104
AD-73873	A-147889	CUGGGCAAGCUGCACAGCAdTdT	735	A-147890	UGCUGUGCAGCUUGCCCAGdTdT	920	CUGGGCAAGCUGCACAGCC	1105
AD-73874	A-147891	UGCACAGCCUGCACCUGGAdTdT	736	A-147892	UCCAGGUGCAGGCUGUGCAdTdT	921	UGCACAGCCUGCACCUGGA	1106
AD-73875	A-147895	CAGCUGCCUGGGACGCAUAdTdT	737	A-147896	UAUGCGUCCCAGGCAGCUGdTdT	922	CAGCUGCCUGGGACGCAUC	1107
AD-73876	A-147897	GACGCAUCCGCCCGCACAAdTdT	738	A-147898	UUGUGCGGGCGGAUGCGUCdTdT	923	GACGCAUCCGCCCGCACAC	1108
AD-73877	A-147899	CGCACACCUUCACCGGCCUdTdT	739	A-147900	AGGCCGGUGAAGGUGUGCGdTdT	924	CGCACACCUUCACCGGCCU	1109
AD-73878	A-147901	UCACCGGCCUCUCGGGGCUdTdT	740	A-147902	AGCCCCGAGAGGCCGGUGAdTdT	925	UCACCGGCCUCUCGGGGCU	1110
AD-73879	A-147903	UCGGGGCUCCGCCGACUCUdTdT	741	A-147904	AGAGUCGGCGGAGCCCCGAdTdT	926	UCGGGGCUCCGCCGACUCU	1111
AD-73880	A-147905	CGACUCUUCCUCAAGGACAdTdT	742	A-147906	UGUCCUUGAGGAAGAGUCGdTdT	927	CGACUCUUCCUCAAGGACA	1112
AD-73881	A-147907	CAAGGACAACGGCCUCGUAdTdT	743	A-147908	UACGAGGCCGUUGUCCUUGdTdT	928	CAAGGACAACGGCCUCGUG	1113
AD-73882	A-147909	GGCCUCGUGGGCAUUGAGAdTdT	744	A-147910	UCUCAAUGCCCACGAGGCCdTdT	929	GGCCUCGUGGGCAUUGAGG	1114
AD-73883	A-147911	UUGAGGAGCAGAGCCUGUAdTdT	745	A-147912	UACAGGCUCUGCUCCUCAAdTdT	930	UUGAGGAGCAGAGCCUGUG	1115
AD-73884	A-147913	AGAGCCUGUGGGGGCUGGAdTdT	746	A-147914	UCCAGCCCCCACAGGCUCUdTdT	931	AGAGCCUGUGGGGGCUGGC	1116
AD-73885	A-147915	GGGCUGGCGGAGCUGCUGAdTdT	747	A-147916	UCAGCAGCUCCGCCAGCCCdTdT	932	GGGCUGGCGGAGCUGCUGG	1117
AD-73886	A-147917	UGCUGGAGCUCGACCUGAAdTdT	748	A-147918	UUCAGGUCGAGCUCCAGCAdTdT	933	UGCUGGAGCUCGACCUGAC	1118
AD-73887	A-147919	GACCUGACCUCCAACCAGAdTdT	749	A-147920	UCUGGUUGGAGGUCAGGUCdTdT	934	GACCUGACCUCCAACCAGC	1119
AD-73888	A-147921	UCCAACCAGCUCACGCACAdTdT	750	A-147922	UGUGCGUGAGCUGGUUGGAdTdT	935	UCCAACCAGCUCACGCACC	1120
AD-73889	A-147923	ACGCACCUGCCCCACCGCAdTdT	751	A-147924	UGCGGUGGGGCAGGUGCGUdTdT	936	ACGCACCUGCCCCACCGCC	1121
AD-73890	A-147925	CACCGCCUCUUCCAGGGCAdTdT	752	A-147926	UGCCCUGGAAGAGGCGGGUGdTdT	937	CACCGCCUCUUCCAGGGCC	1122
AD-73891	A-147927	UCCAGGGCCUGGGCAAGCUdTdT	753	A-147928	AGCUUGCCCAGGCCCUGGAdTdT	938	UCCAGGGCCUGGGCAAGCU	1123
AD-73892	A-147929	GCAAGCUGGAGUACCUGCUdTdT	754	A-147930	AGCAGGUACUCCAGCUUGCdTdT	939	GCAAGCUGGAGUACCUGCU	1124
AD-73893	A-147931	UACCUGCUGCUCUCCCGCAdTdT	755	A-147932	UGCGGGAGAGCAGCAGGUAdTdT	940	UACCUGCUGCUCUCCCGCA	1125
AD-73894	A-147933	CUCUCCCGCAACCGCCUGAdTdT	756	A-147934	UCAGGCGGUUGCGGGAGAGdTdT	941	CUCUCCCGCAACCGCCUGG	1126
AD-73895	A-147935	CCGCCUGGCAGAGCUGCCAdTdT	757	A-147936	UGGCAGCUCUGCCAGGCGGdTdT	942	CCGCCUGGCAGAGCUGCCG	1127
AD-73896	A-147937	AGCUGCCGGCGGACGCCCUdTdT	758	A-147938	AGGGCGUCCGGCCGCAGCUdTdT	943	AGCUGCCGGCGGACGCCCU	1128

Duplex Name	Sense Oligo Name	Sense Oligo Sequence	SEQ ID	Antisense Oligo Name	Antisense Oligo Seq	SEQ ID	mRNA target sequence	SEQ ID
AD-73897	A-147939	GACGCCCUGGGCCCCCUGAdTdT	759	A-147940	UCAGGGGGCCCAGGGCGUCdTdT	944	GACGCCCUGGGCCCCCUGC	1129
AD-73898	A-147941	CCCCUGCAGCGGGCCUUCUdTdT	760	A-147942	AGAAGGCCCGCUGCAGGGCGdTdT	945	CCCCUGCAGCGGGCCUUCU	1130
AD-73899	A-147943	GGGCCUUCUGGCUGGACGUdTdT	761	A-147944	ACGUCCAGCCAGAAGGCCCdTdT	946	GGGCCUUCUGGCUGGACGU	1131
AD-73900	A-147945	UGGACGUCUCGCACAACCAdTdT	762	A-147946	UGGUUGUGCGAGACGUCCAdTdT	947	UGGACGUCUCGCACAACCG	1132
AD-73901	A-147947	ACAACCGCCUGGAGGCAUUdTdT	763	A-147948	AAUGCCUCCAGGCGGUUGUdTdT	948	ACAACCGCCUGGAGGCAUU	1133
AD-73902	A-147949	GAGGCAUUGCCCAACAGCAdTdT	764	A-147950	UGCUGUUGGGCAAUGCCUCdTdT	949	GAGGCAUUGCCCAACAGCC	1134
AD-73903	A-147951	CAACAGCCUCUUGGCACCAdTdT	765	A-147952	UGGUGCCAAGAGGCUGUUGdTdT	950	CAACAGCCUCUUGGCACCA	1135
AD-73904	A-147953	UUGGCACCACUGGGGCGGAdTdT	766	A-147954	UCCGCCCCAGUGGUGCCAAdTdT	951	UUGGCACCACUGGGGCGGC	1136
AD-73905	A-147955	UGGGGCGGCUGCGCUACCUdTdT	767	A-147956	AGGUAGCGCAGCCGCCCCAdTdT	952	UGGGGCGGCUGCGCUACCU	1137
AD-73906	A-147957	CGCUACCUCAGCCUCAGGAdTdT	768	A-147958	UCCUGAGGCUGAGGUAGCGdTdT	953	CGCUACCUCAGCCUCAGGA	1138
AD-73907	A-147959	UCAGGAACAACUCACUGCAdTdT	769	A-147960	UGCAGUGAGUUGUUCCUGAdTdT	954	UCAGGAACAACUCACUGCG	1139
AD-73908	A-147961	CUCACUGCGGACCUUCACAdTdT	770	A-147962	UGUGAAGGUCCGCAGUGAGdTdT	955	CUCACUGCGGACCUUCACG	1140
AD-73909	A-147963	ACCUUCACGCCGCAGCCCAdTdT	771	A-147964	UGGGCUGCGGCGUGAAGGUdTdT	956	ACCUUCACGCCGCAGCCCC	1141
AD-73910	A-147965	CAGCCCCCGGGCCUGGAGAdTdT	772	A-147966	UCUCCAGGCCCGGGGGGCUGdTdT	957	CAGCCCCCGGGCCUGGAGC	1142
AD-73911	A-147967	GCCUGGAGCGCCUGUGGCUdTdT	773	A-147968	AGCCACAGGCGCUCCAGGCdTdT	958	GCCUGGAGCGCCUGUGGCU	1143
AD-73912	A-147969	CUGUGGCUGGAGGGUAACAdTdT	774	A-147970	UGUUACCCUCCAGCCACAGdTdT	959	CUGUGGCUGGAGGGUAACC	1144
AD-73913	A-147971	GGUAACCCCUGGGACUGUAdTdT	775	A-147972	UACAGUCCCAGGGGUUACCdTdT	960	GGUAACCCCUGGGACUGUG	1145
AD-73914	A-147973	GGGACUGUGGCUGCCCUCUdTdT	776	A-147974	AGAGGGCAGCCACAGUCCCdTdT	961	GGGACUGUGGCUGCCCUCU	1146
AD-73915	A-147975	UGCCCUCUCAAGGCGCUGAdTdT	777	A-147976	UCAGCGCCUUGAGAGGGCAdTdT	962	UGCCCUCUCAAGGCGCUGC	1147
AD-73916	A-147977	CGCUGCGGGACUUCGCCCUdTdT	778	A-147978	AGGGCGAAGUCCCGCAGCGdTdT	963	CGCUGCGGGACUUCGCCCU	1148
AD-73917	A-147979	UUCGCCCUGCAGAACCCCAdTdT	779	A-147980	UGGGGUUCUGCAGGGCGAAdTdT	964	UUCGCCCUGCAGAACCCCA	1149
AD-73918	A-147981	CAGAACCCCAGUGCUGUGAdTdT	780	A-147982	UCACAGCACUGGGGUUCUGdTdT	965	CAGAACCCCAGUGCUGUGC	1150
AD-73919	A-147983	UGCUGUGCCCCGCUUCGUAdTdT	781	A-147984	UACGAAGCGGGGCACAGCAdTdT	966	UGCUGUGCCCCGCUUCGUC	1151
AD-73920	A-147985	CUUCGUCCAGGCCAUCUGUdTdT	782	A-147986	ACAGAUGGCCUGGACGAAGdTdT	967	CUUCGUCCAGGCCAUCUGU	1152
AD-73921	A-147987	CAUCUGUGAGGGGGACGAUdTdT	783	A-147988	AUCGUCCCCCUCACAGAUGdTdT	968	CAUCUGUGAGGGGGACGAU	1153
AD-73922	A-147989	GGGGACGAUUGCCAGCCGAdTdT	784	A-147990	UCGGCUGGCAAUCGUCCCCdTdT	969	GGGGACGAUUGCCAGCCGC	1154
AD-73923	A-147991	CAGCCGCCCGCGUACACCUdTdT	785	A-147992	AGGUGUACGCGGGCGGGCUGdTdT	970	CAGCCGCCCGCGUACACCU	1155

Duplex Name	Sense Oligo Name	Sense Oligo Sequence	SEQ ID	Antisense Oligo Name	Antisense Oligo Seq	SEQ ID	mRNA target sequence	SEQ ID
AD-73924	A-147993	CGUACACCUACAACAACAACAUdTdT	786	A-147994	AUGUUGUUGUAGGUGUACGdTdT	971	CGUACACCUACAACAACAU	1156
AD-73925	A-147995	AACAACAUCACCUGUGCCAdTdT	787	A-147996	UGGCACAGGUGAUGUUGUUdTdT	972	AACAACAUCACCUGUGCCA	1157
AD-73926	A-147997	UGUGCCAGCCCGCCCGAGAdTdT	788	A-147998	UCUCGGGCGGGCUGGCACAdTdT	973	UGUGCCAGCCCGCCCGAGG	1158
AD-73927	A-147999	CGCCCGAGGUCGUGGGGCUdTdT	789	A-148000	AGCCCCACGACCUCGGGCGdTdT	974	CGCCCGAGGUCGUGGGGCU	1159
AD-73928	A-148001	CGUGGGGCUCGACCUGCGAdTdT	790	A-148002	UCGCAGGUCGAGCCCCACGdTdT	975	CGUGGGGCUCGACCUGCGG	1160
AD-73929	A-148003	ACCUGCGGGACCUCAGCGAdTdT	791	A-148004	UCGCUGAGGUCCCCGCAGGUdTdT	976	ACCUGCGGGACCUCAGCGA	1161
AD-73930	A-148005	UCAGCGAGGCCCACUUUGAdTdT	792	A-148006	UCAAAGUGGGCCUCGCUGAdTdT	977	UCAGCGAGGCCCACUUUGC	1162
AD-73931	A-148007	ACUUUGCUCCCUGCUGACAdTdT	793	A-148008	UGUCAGCAGGGAGCAAAGUdTdT	978	ACUUUGCUCCCUGCUGACC	1163
AD-73932	A-148009	CCUGCUGACCAGGUCCCCAdTdT	794	A-148010	UGGGGACCUGGUCAGCAGGdTdT	979	CCUGCUGACCAGGUCCCCG	1164
AD-73933	A-148011	UCCCCGGACUCAAGCCCCAdTdT	795	A-148012	UGGGGCUUGAGUCCGGGGAdTdT	980	UCCCCGGACUCAAGCCCCG	1165
AD-73934	A-148013	CAAGCCCCGGACUCAGGCAdTdT	796	A-148014	UGCCUGAGUCCGGGGGCUUGdTdT	981	CAAGCCCCGGACUCAGGCC	1166
AD-73935	A-148015	UCAGGCCCCCACCUGGCUAdTdT	797	A-148016	UAGCCAGGUGGGGGGCCUGAdTdT	982	UCAGGCCCCCACCUGGCUC	1167
AD-73936	A-148017	ACCUGGCUCACCUUGUGCUdTdT	798	A-148018	AGCACAAGGUGAGCCAGGUdTdT	983	ACCUGGCUCACCUUGUGCU	1168
AD-73937	A-148019	UUGUGCUGGGGACAGGUCAdTdT	799	A-148020	UGACCUGUCCCCAGCACAAdTdT	984	UUGUGCUGGGGACAGGUCC	1169
AD-73938	A-148021	GACAGGUCCUCAGUGUCCUdTdT	800	A-148022	AGGACACUGAGGACCUGUCdTdT	985	GACAGGUCCUCAGUGUCCU	1170
AD-73939	A-148023	CAGUGUCCUCAGGGGCCUAdTdT	801	A-148024	UAGGCCCCUGAGGACACUGdTdT	986	CAGUGUCCUCAGGGGCCUG	1171
AD-73940	A-148025	GGGCCUGCCCAGUGCACUUdTdT	802	A-148026	AAGUGCACUGGGCAGGCCCdTdT	987	GGGCCUGCCCAGUGCACUU	1172
AD-73941	A-148027	UGCACUUGCUGGAAGACGAdTdT	803	A-148028	UCGUCUUCCAGCAAGUGCAdTdT	988	UGCACUUGCUGGAAGACGC	1173
AD-73942	A-148029	UGGAAGACGCAAGGGCCUAdTdT	804	A-148030	UAGGCCCUUGCGUCUUCCAdTdT	989	UGGAAGACGCAAGGGCCUG	1174
AD-73943	A-148031	AGGGCCUGAUGGGGUGGAAdTdT	805	A-148032	UUCCACCCCAUCAGGCCCUdTdT	990	AGGGCCUGAUGGGGUGGAA	1175
AD-73944	A-148033	GGGUGGAAGGCAUGGCGGAdTdT	806	A-148034	UCCGCCAUGCCUUCCACCCdTdT	991	GGGUGGAAGGCAUGGCGGC	1176
AD-73945	A-148035	UGGCGGCCCCCCCCAGCUGUdTdT	807	A-148036	ACAGCUGGGGGGGGCCGCCAdTdT	992	UGGCGGCCCCCCCCAGCUGU	1177
AD-73946	A-148037	CAGCUGUCAUCAAUUAAAGdTdT	808	A-148038	CUUUAAUUGAUGACAGCUGdTdT	993	CAGCUGUCAUCAAUUAAAG	1178
AD-73947	A-148039	AAUUAAAGGCAAAGGCAAUdTdT	809	A-148040	AUUGCCUUUGCCUUUAAUUdTdT	994	AAUUAAAGGCAAAGGCAAU	1179
AD-73948	A-148041	AAGGCAAUCGAAUCUAAAAAdTdT	810	A-148042	UUUUAGAUUCGAUUGCCUUdTdT	995	AAGGCAAUCGAAUCUAAAAA	1180

Example 5 - *In vitro* screening

Cell culture and plasmids/transfections for Dual-Glo® assay:

HeLa cells (ATCC) were transfected by adding 4.9µl 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. Fortyµl of Dulbecco's Modified Eagle Medium (Life Tech) containing ~5 $x10^3$ cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10nM and 0.1nM.

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, 50µl of Lysis/Binding Buffer and 25µl of lysis buffer containing 3µl 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 150µl Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150µl Elution Buffer, re-captured and supernatant removed.

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

Ten µl of a master mix containing 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl 10x Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H2O per reaction was added to RNA isolated above. Plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2 hours at 37°C.

Real time PCR

Two µl of cDNA were added to a master mix containing 0.5µl of Human GAPDH TaqMan Probe (4326317E), 0.5µl IGF-1 human probe (Hs01547656_m1) and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) 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 in duplicate 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.

Table 9. Unmodified Sense and Antisense Strand Sequences of IGF-1 dsRNAs

Duplex Name	Sense Oligo Name	Sense sequence	Range in SEQ ID No:11	SEQ ID NO	Antisense Oligo Name	Antisense sequence	Range in SEQ ID No:11	SEQ ID NO
AD-66716	A-133440	GCUGCUUCCGGAGCUGUGAUA	548-568	1181	A-133441	UAUCACAGCUCCGGAAGCAGCAC	546-568	1247
AD-66717	A-133442	UCUGCGGGGCUGAGCUGGUGA	422-442	1182	A-133443	UCACCAGCUCAGCCCCGCAGAGC	420-442	1248
AD-66718	A-133444	CCUGCUCACCUUCACCAGCUA	378-398	1183	A-133445	UAGCUGGUGAAGGUGAGCAGGCA	376-398	1249
AD-66719	A-133446	GUGGAGACAGGGGCUUUUAUU	461-481	1184	A-133447	AAUAAAAGCCCCUGUCUCCACAC	459-481	1250
AD-66720	A-133448	UGGAGACAGGGGCUUUUAUUU	462-482	1185	A-133449	AAAUAAAAGCCCCUGUCUCCACA	460-482	1251
AD-66721	A-133450	GAGACAGGGGCUUUUAUUUCA	464-484	1186	A-133451	UGAAAUAAAAGCCCCUGUCUCCA	462-484	1252
AD-66722	A-133452	CAUGUCCUCCUCGCAUCUCUU	342-362	1187	A-133453	AAGAGAUGCGAGGAGGACAUGGU	340-362	1253
AD-66723	A-133454	UUUUAUUUCAACAAGCCCACA	475-495	1188	A-133455	UGUGGGCUUGUUGAAAUAAAAGC	473-495	1254
AD-66724	A-133456	UGUGGAGACAGGGGCUUUUAU	460-480	1189	A-133457	AUAAAAGCCCCUGUCUCCACACA	458-480	1255
AD-66725	A-133458	UGGAUGAGUGCUGCUUCCGGA	539-559	1190	A-133459	UCCGGAAGCAGCACUCAUCCACG	537-559	1256
AD-66726	A-133460	UCGUGUGUGGAGACAGGGGCU	455-475	1191	A-133461	AGCCCCUGUCUCCACACACGAAC	453-475	1257
AD-66727	A-133462	GAUGUAUUGCGCACCCCUCAA	582-602	1192	A-133463	UUGAGGGGUGCGCAAUACAUCUC	580-602	1258
AD-66728	A-133464	UUCAGUUCGUGUGUGGAGACA	449-469	1193	A-133465	UGUCUCCACACACGAACUGAAGA	447-469	1259
AD-66729	A-133466	CUCCUCGCAUCUCUUCUACCU	348-368	1194	A-133467	AGGUAGAAGAGAUGCGAGGAGGA	346-368	1260
AD-66730	A-133468	AGAUGUAUUGCGCACCCCUCA	581-601	1195	A-133469	UGAGGGGUGCGCAAUACAUCUCC	579-601	1261
AD-66731	A-133470	GCCACACCGACAUGCCCAAGA	638-658	1196	A-133471	UCUUGGGCAUGUCGGUGUGGCGC	636-658	1262
AD-66732	A-133472	GGAGAUGUAUUGCGCACCCCU	579-599	1197	A-133473	AGGGGUGCGCAAUACAUCUCCAG	577-599	1263
AD-66733	A-133474	UUCAACAAGCCCACAGGGUAU	481-501	1198	A-133475	AUACCCUGUGGGCUUGUUGAAAU	479-501	1264
AD-66734	A-133476	UGCCCAGCGCCACACCGACAU	630-650	1199	A-133478	AUGUCGGUGUGGCGCUGGGCACG	628-650	1265
AD-66735	A-133480	GCAUCGUGGAUGAGUGCUGCU	533-553	1200	A-133482	AGCAGCACUCAUCCACGAUGCCU	531-553	1266
AD-66736	A-133484	GGAGACAGGGGCUUUUAUUUA	463-483	1201	A-133486	UAAAUAAAAGCCCCUGUCUCCAC	461-483	1267
AD-66737	A-133488	GGGCUUUUAUUUCAACAAGCA	471-491	1202	A-133490	UGCUUGUUGAAAUAAAAGCCCCU	469-491	1268
AD-66738	A-133492	UCGUGGAUGAGUGCUGCUUCA	536-556	1203	A-133494	UGAAGCAGCACUCAUCCACGAUG	534-556	1269

Duplex Name	Sense Oligo Name	Sense sequence	Range in SEQ ID No:11	SEQ ID NO	Antisense Oligo Name	Antisense sequence	Range in SEQ ID No:11	SEQ ID NO
AD-66739	A-133496	UCCUCGCAUCUCUUCUACCUA	349-369	1204	A-133498	UAGGUAGAAGAGAUGCGAGGAGG	347-369	1270
AD-66740	A-133500	GGGGCUUUUAUUUCAACAAGA	470-490	1205	A-133502	UCUUGUUGAAAUAAAAGCCCCUG	468-490	1271
AD-66741	A-133504	UUUAUUUCAACAAGCCCCACAA	476-496	1206	A-133506	UUGUGGGCUUGUUGAAAUAAAAG	474-496	1272
AD-66742	A-133508	GCUGGAGAUGUAUUGCGCACA	576-596	1207	A-133510	UGUGCGCAAUACAUCUCCAGCCU	574-596	1273
AD-66743	A-133512	GGGUAUGGCUCCCAGCAGUCGA	496-516	1208	A-133513	UCGACUGCUGGAGCCAUACCCUG	494-516	1274
AD-66744	A-133514	CUGGAGAUGUAUUGCGCACCA	577-597	1209	A-133515	UGGUGCGCAAUACAUCUCCAGCC	575-597	1275
AD-66745	A-133516	GAAGAUGCACACCAUGUCCUA	330-350	1210	A-133517	UAGGACAUGGUGUGCAUCUUCAC	328-350	1276
AD-66746	A-133477	GAUGCUCUUCAGUUCGUGUGU	442-462	1211	A-133479	ACACACGAACUGAAGAGCAUCCA	440-462	1277
AD-66747	A-133481	UGGAUGCUCUUCAGUUCGUGU	440-460	1212	A-133483	ACACGAACUGAAGAGCAUCCACC	438-460	1278
AD-66748	A-133485	GGUGGAUGCUCUUCAGUUCGU	438-458	1213	A-133487	ACGAACUGAAGAGCAUCCACCAG	436-458	1279
AD-66749	A-133489	UGAGCUGGUGGAUGCUCUUCA	432-452	1214	A-133491	UGAAGAGCAUCCACCAGCUCAGC	430-452	1280
AD-66750	A-133493	GCUGGUGGAUGCUCUUCAGUU	435-455	1215	A-133495	AACUGAAGAGCAUCCACCAGCUC	433-455	1281
AD-66751	A-133497	GGAUGCUCUUCAGUUCGUGUA	441-461	1216	A-133499	UACACGAACUGAAGAGCAUCCAC	439-461	1282
AD-66752	A-133501	CUGGUGGAUGCUCUUCAGUUA	436-456	1217	A-133503	UAACUGAAGAGCAUCCACCAGCU	434-456	1283
AD-66753	A-133505	GGCUGAGCUGGUGGAUGCUCU	429-449	1218	A-133507	AGAGCAUCCACCAGCUCAGCCCC	427-449	1284
AD-66754	A-133509	CUGAGCUGGUGGAUGCUCUUA	431-451	1219	A-133511	UAAGAGCAUCCACCAGCUCAGCC	429-451	1285
AD-66755	A-133518	CAUUGUGGAUGAGUGUUGCUU	534-554	1220	A-133519	AAGCAACACUCAUCCACAAUGCC	532-554	1286
AD-66756	A-133520	AGAUACACAUCAUGUCGUCUU	*	1221	A-133521	AAGACGACAUGAUGUGUAUCUUU	*	1287
AD-66757	A-133522	UGGAUGAGUGUUGCUUCCGGA	539-559	1222	A-133523	UCCGGAAGCAACACUCAUCCACA	537-559	1288
AD-66758	A-133524	UGUUGCUUCCGGAGCUGUGAU	547-567	1223	A-133525	AUCACAGCUCCGGAAGCAACACU	545-567	1289
AD-66759	A-133526	GCUUUUACUUCAACAAGCCCA	473-493	1224	A-133527	UGGGCUUGUUGAAGUAAAAGCCC	471-493	1290
AD-66760	A-133528	AUGAGUGUUGCUUCCGGAGCU	542-562	1225	A-133529	AGCUCCGGAAGCAACACUCAUCC	540-562	1291
AD-66761	A-133530	CACACUGACAUGCCCAAGACU	640-660	1226	A-133531	AGUCUUGGGCAUGUCAGUGUGGC	638-660	1292
AD-66762	A-133532	GCUAUGGCUCCAGCAUUCGGA	497-517	1227	A-133533	UCCGAAUGCUGGAGCCAUAGCCU	495-517	1293

Duplex Name	Sense Oligo Name	Sense sequence	Range in SEQ ID No:11	SEQ ID NO	Antisense Oligo Name	Antisense sequence	Range in SEQ ID No:11	SEQ ID NO
AD-66763	A-133534	AAGAUACACAUCAUGUCGUCU	*	1228	A-133535	AGACGACAUGAUGUGUAUCUUUA	*	1294
AD-66764	A-133536	UUGCUUCCGGAGCUGUGAUCU	549-569	1229	A-133537	AGAUCACAGCUCCGGAAGCAACA	547-569	1295
AD-66765	A-133538	UCCGGAGCUGUGAUCUGAGGA	554-574	1230	A-133539	UCCUCAGAUCACAGCUCCGGAAG	552-574	1296
AD-66766	A-133540	GUGGAUGAGUGUUGCUUCCGA	538-558	1231	A-133541	UCGGAAGCAACACUCAUCCACAA	536-558	1297
AD-66767	A-133542	UACACAUCAUGUCGUCUUCAA	*	1232	A-133543	UUGAAGACGACAUGAUGUGUAUC	*	1298
AD-66768	A-133544	AAAGAUACACAUCAUGUCGUA	*	1233	A-133545	UACGACAUGAUGUGUAUCUUUAU	*	1299
AD-66769	A-133546	GGCUAUGGCUCCAGCAUUCGA	496-516	1234	A-133547	UCGAAUGCUGGAGCCAUAGCCUG	494-516	1300
AD-66770	A-133548	ACACUGACAUGCCCAAGACUA	641-661	1235	A-133549	UAGUCUUGGGCAUGUCAGUGUGG	639-661	1301
AD-66771	A-133550	AGUGUUGCUUCCGGAGCUGUA	545-565	1236	A-133551	UACAGCUCCGGAAGCAACACUCA	543-565	1302
AD-66772	A-133552	GAGACCCUUUGCGGGGCUGAA	*	1237	A-133553	UUCAGCCCCGCAAAGGGUCUCUG	*	1303
AD-66773	A-133554	ACUGACAUGCCCAAGACUCAA	643-663	1238	A-133555	UUGAGUCUUGGGCAUGUCAGUGU	641-663	1304
AD-66774	A-133556	GAUACACAUCAUGUCGUCUUA	*	1239	A-133557	UAAGACGACAUGAUGUGUAUCUU	*	1305
AD-66775	A-133558	AAGCCCACAGGCUAUGGCUCA	487-507	1240	A-133559	UGAGCCAUAGCCUGUGGGCUUGU	485-507	1306
Duplex Name	**Sense Oligo Name**	**Sense sequence**	**Range in SEQ ID No:17**	**SEQ ID NO**	**Antisense Oligo Name**	**Antisense sequence**	**Range in SEQ ID NO:17**	**SEQ ID NO**
AD-66756	A-133520	AGAUACACAUCAUGUCGUCUU	366-368	1241	A-133521	AAGACGACAUGAUGUGUAUCUUU	364-368	1307
AD-66763	A-133534	AAGAUACACAUCAUGUCGUCU	365-385	1242	A-133535	AGACGACAUGAUGUGUAUCUUUA	363-385	1308
AD-66767	A-133542	UACACAUCAUGUCGUCUUCAA	369-389	1243	A-133543	UUGAAGACGACAUGAUGUGUAUC	367-389	1309
AD-66768	A-133544	AAAGAUACACAUCAUGUCGUA	364-384	1244	A-133545	UACGACAUGAUGUGUAUCUUUAU	362-384	1310
AD-66772	A-133552	GAGACCCUUUGCGGGGCUGAA	449-469	1245	A-133553	UUCAGCCCCGCAAAGGGUCUCUG	447-469	1311
AD-66774	A-133556	GAUACACAUCAUGUCGUCUUA	367-387	1246	A-133557	UAAGACGACAUGAUGUGUAUCUU	365-387	1312

* Targeting sequence in NM_010512 (SEQ ID NO: 7).

Table 10. IGF-1 Screen in HeLa cells

Each duplex was tested in duplicate and data were normalized to cells transfected with a non-targeting control siRNA AD-1955.

Duplex ID	10nM Avg	STDEV	0.1nM Avg	STDEV
AD-66716	58.4	3.1	90.3	24.0
AD-66717	82.2	1.6	81.1	10.3
AD-66718	62.7	7.0	76.4	10.1
AD-66719	51.0	6.7	83.2	8.1
AD-66720	30.3	0.9	74.9	15.7
AD-66721	58.0	8.5	82.5	23.9
AD-66722	4.7	1.0	29.2	9.0
AD-66723	70.5	7.3	85.4	11.3
AD-66724	32.4	8.0	82.7	9.7
AD-66725	22.6	6.4	72.8	12.1
AD-66726	32.8	2.1	76.4	25.0
AD-66727	53.5	1.1	83.1	6.5
AD-66728	59.1	11.5	91.5	15.2
AD-66729	27.9	4.2	75.3	27.4
AD-66730	79.1	12.7	87.8	18.4
AD-66731	86.4	15.9	97.0	25.4
AD-66732	81.0	8.3	80.9	15.0
AD-66733	8.7	3.7	43.7	1.9
AD-66734	65.4	3.2	83.7	15.5
AD-66735	62.0	6.7	82.6	8.1
AD-66736	71.9	4.5	83.5	23.4
AD-66737	68.7	4.0	92.0	14.4
AD-66738	19.2	3.3	79.4	14.3
AD-66739	10.6	2.7	61.8	23.6
AD-66740	23.2	4.4	68.2	14.6
AD-66741	83.6	0.5	76.5	14.2
AD-66742	73.1	2.2	86.2	10.1
AD-66743	58.9	1.8	88.1	11.2
AD-66744	53.9	2.1	96.8	7.6
AD-66745	28.3	5.5	76.8	7.9
AD-66746	6.3	0.7	50.1	3.9
AD-66747	8.5	2.8	50.0	0.0
AD-66748	6.2	1.3	34.8	4.1
AD-66749	30.0	0.5	92.4	3.2
AD-66750	27.6	0.7	74.8	1.5
AD-66751	50.1	0.3	89.3	15.2
AD-66752	9.6	1.3	55.5	12.4
AD-66753	54.6	2.1	89.0	0.9
AD-66754	78.6	16.8	104.3	2.0

Duplex ID	10nM Avg	STDEV	0.1nM Avg	STDEV
AD-66755	46.8	4.8	103.4	18.6
AD-66756	86.3	2.5	95.9	18.2
AD-66757	69.1	0.7	103.0	5.0
AD-66758	67.5	3.6	86.5	2.5
AD-66759	106.5	16.2	91.4	20.4
AD-66760	54.2	1.6	86.8	0.4
AD-66761	40.8	3.8	92.2	7.2
AD-66762	96.8	7.1	100.6	8.4
AD-66763	81.2	11.4	92.1	0.0
AD-66764	86.0	2.2	101.2	12.9
AD-66765	100.9	13.7	93.2	25.3
AD-66766	36.6	3.9	78.5	15.7
AD-66767	124.0	12.2	89.9	1.3
AD-66768	113.9	15.1	92.7	15.4
AD-66769	92.0	7.1	93.6	8.2
AD-66770	79.7	3.6	98.7	1.9
AD-66771	60.6	12.1	97.6	10.5
AD-66772	95.5	7.0	95.9	9.9
AD-66773	61.3	3.9	90.7	7.5
AD-66774	95.6	8.9	81.9	21.8
AD-66775	113.1	13.9	99.5	6.8
AD-1955	100.0	8.0		

Table 11. Modified Sense and Antisense Sequences of IGF-1

Duplex Name	Sense Oligo Name	Modified Sense Sequence	SEQ ID NO	Antisense Oligo Name	Modified Antisense Sequence	SEQ ID NO:
AD-66716	A-133440	GfscsUfgCfuUfcCfGfGfaGfcUfgUfgAfuAfL96	1313	A-133441	usAfsuCfaCfaGfcUfccgGfaAfgCfaGfcsasc	1373
AD-66717	A-133442	UfscsUfgCfgGfgGfCfUfgAfgCfuGfgUfgAfL96	1314	A-133443	usCfsaCfcAfgCfuCfagcCfcCfgCfaGfasgsc	1374
AD-66718	A-133444	CfscsUfgCfuCfaCfCfUfuCfaCfcAfgCfuAfL96	1315	A-133445	usAfsgCfuGfgUfgAfaggUfgAfgCfaGfgscsa	1375
AD-66719	A-133446	GfsusGfgAfgAfcAfGfGfgGfcUfuUfuAfuUfL96	1316	A-133447	asAfsuAfaAfaGfcCfccuGfuCfuCfcAfcsasc	1376
AD-66720	A-133448	UfsgsGfaGfaCfaGfGfGfgCfuUfuUfaUfuUfL96	1317	A-133449	asAfsaUfaAfaAfgCfcccUfgUfcUfcCfascsa	1377
AD-66721	A-133450	GfsasGfaCfaGfgGfGfCfuUfuUfaUfuUfcAfL96	1318	A-133451	usGfsaAfaUfaAfaAfgccCfcUfgUfcUfcscsa	1378
AD-66722	A-133452	CfsasUfgUfcCfuCfCfUfcGfcAfuCfuCfuUfL96	1319	A-133453	asAfsgAfgAfuGfcGfaggAfgGfaCfaUfgsgsu	1379
AD-66723	A-133454	UfsusUfuAfuUfuCfAfAfcAfaGfcCfcAfcAfL96	1320	A-133455	usGfsuGfgGfcUfuGfuugAfaAfuAfaAfasgsc	1380
AD-66724	A-133456	UfsgsUfgGfaGfaCfAfGfgGfgCfuUfuUfaUfL96	1321	A-133457	asUfsaAfaAfgCfcCfcugUfcUfcCfaCfascsa	1381
AD-66725	A-133458	UfsgsGfaUfgAfgUfGfCfuGfcUfuCfcGfgAfL96	1322	A-133459	usCfscGfgAfaGfcAfgcaCfuCfaUfcCfascsg	1382
AD-66726	A-133460	UfscsGfuGfuGfuGfGfAfgAfcAfgGfgGfcUfL96	1323	A-133461	asGfscCfcCfuGfuCfuccAfcAfcAfcGfasasc	1383
AD-66727	A-133462	GfsasUfgUfaUfuGfCfGfcAfcCfcCfuCfaAfL96	1324	A-133463	usUfsgAfgGfgGfuGfcgcAfaUfaCfaUfcsusc	1384
AD-66728	A-133464	UfsusCfaGfuUfcGfUfGfuGfuGfgAfgAfcAfL96	1325	A-133465	usGfsuCfuCfcAfcAfcacGfaAfcUfgAfasgsa	1385
AD-66729	A-133466	CfsusCfcUfcGfcAfUfCfuCfuUfcUfaCfcUfL96	1326	A-133467	asGfsgUfaGfaAfgAfgauGfcGfaGfgAfgsgsa	1386
AD-66730	A-133468	AfsgsAfuGfuAfuUfGfCfgCfaCfcCfcUfcAfL96	1327	A-133469	usGfsaGfgGfgUfgCfgcaAfuAfcAfuCfuscsc	1387
AD-66731	A-133470	GfscsCfaCfaCfcGfAfCfaUfgCfcCfaAfgAfL96	1328	A-133471	usCfsuUfgGfgCfaUfgucGfgUfgUfgGfcsgsc	1388
AD-66732	A-133472	GfsgsAfgAfuGfuAfUfUfgCfgCfaCfcCfcUfL96	1329	A-133473	asGfsgGfgUfgCfgCfaauAfcAfuCfuCfcsasg	1389
AD-66733	A-133474	UfsusCfaAfcAfaGfCfCfcAfcAfgGfgUfaUfL96	1330	A-133475	asUfsaCfcCfuGfuGfggcUfuGfuUfgAfasasu	1390
AD-66734	A-133476	UfsgsCfcCfaGfcGfCfCfaCfaCfcGfaCfaUfL96	1331	A-133478	asUfsgUfcGfgUfgUfggcGfcUfgGfgCfascsg	1391
AD-66735	A-133480	GfscsAfuCfgUfgGfAfUfgAfgUfgCfuGfcUfL96	1332	A-133482	asGfscAfgCfaCfuCfaucCfaCfgAfuGfcscsu	1392
AD-66736	A-133484	GfsgsAfgAfcAfgGfGfGfcUfuUfuAfuUfuAfL96	1333	A-133486	usAfsaAfuAfaAfaGfcccCfuGfuCfuCfcsasc	1393
AD-66737	A-133488	GfsgsGfcUfuUfuAfUfUfuCfaAfcAfaGfcAfL96	1334	A-133490	usGfscUfuGfuUfgAfaauAfaAfaGfcCfcscsu	1394
AD-66738	A-133492	UfscsGfuGfgAfuGfAfGfuGfcUfgCfuUfcAfL96	1335	A-133494	usGfsaAfgCfaGfcAfcucAfuCfcAfcGfasusg	1395
AD-66739	A-133496	UfscsCfuCfgCfaUfCfUfcUfuCfuAfcCfuAfL96	1336	A-133498	usAfsgGfuAfgAfaGfagaUfgCfgAfgGfasgsg	1396

Duplex Name	Sense Oligo Name	Modified Sense Sequence	SEQ ID NO	Antisense Oligo Name	Modified Antisense Sequence	SEQ ID NO:
AD-66740	A-133500	GfsgsGfgCfuUfuUfAfUfuUfcAfaCfaAfgAfL96	1337	A-133502	usCfsuUfgUfuGfaAfauaAfaAfgCfcCfcsusg	1397
AD-66741	A-133504	UfsusUfaUfuUfcAfAfCfaAfgCfcCfaCfaAfL96	1338	A-133506	usUfsgUfgGfgCfuUfguuGfaAfaUfaAfasasg	1398
AD-66742	A-133508	GfscsUfgGfaGfaUfGfUfaUfuGfcGfcAfcAfL96	1339	A-133510	usGfsuGfcGfcAfaUfacaUfcUfcCfaGfcscsu	1399
AD-66743	A-133512	GfsgsGfuAfuGfgCfUfCfcAfgCfaGfuCfgAfL96	1340	A-133513	usCfsgAfcUfgCfuGfgagCfcAfuAfcCfcusg	1400
AD-66744	A-133514	CfsusGfgAfgAfuGfUfAfuUfgCfgCfaCfcAfL96	1341	A-133515	usGfsgUfgCfgCfaAfuacAfuCfuCfcAfgscsc	1401
AD-66745	A-133516	GfsasAfgAfuGfcAfCfAfcCfaUfgUfcCfuAfL96	1342	A-133517	usAfsgGfaCfaUfgGfguguGfcAfuCfuUfcsasc	1402
AD-66746	A-133477	GfsasUfgCfuCfuUfCfAfgUfuCfgUfgUfgUfL96	1343	A-133479	asCfsaCfaCfgAfaCfugaAfgAfgAfgCfaUfcscsa	1403
AD-66747	A-133481	UfsgsGfaUfgCfuCfUfUfcAfgUfuCfgUfgUfL96	1344	A-133483	asCfsaCfgAfaCfuGfaagAfgCfaUfcCfascsc	1404
AD-66748	A-133485	GfsgsUfgGfaUfgCfUfCfuUfcAfgUfuCfgUfL96	1345	A-133487	asCfsgAfaCfuGfaAfgagCfaUfcCfaCfcsasg	1405
AD-66749	A-133489	UfsgsAfgCfuGfgUfGfGfaUfgCfuCfuUfcAfL96	1346	A-133491	usGfsaAfgAfgCfaUfccaCfcAfgCfuCfasgsc	1406
AD-66750	A-133493	GfscsUfgGfuGfgAfUfGfcUfcUfuCfaGfuUfL96	1347	A-133495	asAfscUfgAfaGfaGfcauCfcAfcCfaGfcsusc	1407
AD-66751	A-133497	GfsgsAfuGfcUfcUfUfCfaGfuUfcGfuGfuAfL96	1348	A-133499	usAfscAfcGfaAfcUfgaaGfaGfcAfuCfcsasc	1408
AD-66752	A-133501	CfsusGfgUfgGfaUfGfCfuCfuUfcAfgUfuAfL96	1349	A-133503	usAfsaCfuGfaAfgAfgcaUfcCfaCfcAfgscsu	1409
AD-66753	A-133505	GfsgsCfuGfaGfcUfGfGfuGfgAfuGfcUfcUfL96	1350	A-133507	asGfsaGfcAfuCfcAfccaGfcUfcAfgCfcscsc	1410
AD-66754	A-133509	CfsusGfaGfcUfgGfUfGfgAfuGfcUfcUfuAfL96	1351	A-133511	usAfsaGfaGfcAfuCfcacCfaGfcUfcAfgscsc	1411
AD-66755	A-133518	CfsasUfuGfuGfgAfUfGfaGfuGfuUfgCfuUfL96	1352	A-133519	asAfsgCfaAfcAfcUfcauCfcAfcAfaUfgscsc	1412
AD-66756	A-133520	AfsgsAfuAfcAfcAfUfCfaUfgUfcGfuCfuUfL96	1353	A-133521	asAfsgAfcGfaCfaUfgauGfuGfuAfuCfususu	1413
AD-66757	A-133522	UfsgsGfaUfgAfgUfGfUfuGfcUfuCfcGfgAfL96	1354	A-133523	usCfscGfgAfaGfcAfacaCfuCfaUfcCfascsa	1414
AD-66758	A-133524	UfsgsUfuGfcUfuCfCfGfgAfgCfuGfuGfaUfL96	1355	A-133525	asUfscAfcAfgCfuCfcggAfaGfcAfaCfascsu	1415
AD-66759	A-133526	GfscsUfuUfuAfcUfUfCfaAfcAfaGfcCfcAfL96	1356	A-133527	usGfsgGfcUfuGfuUfgaaGfuAfaAfaGfcscsc	1416
AD-66760	A-133528	AfsusGfaGfuGfuUfGfCfuUfcCfgGfaGfcUfL96	1357	A-133529	asGfscUfcCfgGfaAfgcaAfcAfcUfcAfuscsc	1417
AD-66761	A-133530	CfsasCfaCfuGfaCfAfUfgCfcCfaAfgAfcUfL96	1358	A-133531	asGfsuCfuUfgGfgCfaugUfcAfgUfgUfgsgsc	1418
AD-66762	A-133532	GfscsUfaUfgGfcUfCfCfaGfcAfuUfcGfgAfL96	1359	A-133533	usCfscGfaAfuGfcUfggaGfcCfaUfaGfcscsu	1419
AD-66763	A-133534	AfsasGfaUfaCfaCfAfUfcAfuGfuCfgUfcUfL96	1360	A-133535	asGfsaCfgAfcAfuGfaugUfgUfaUfcUfususa	1420
AD-66764	A-133536	UfsusGfcUfuCfcGfGfAfgCfuGfuGfaUfcUfL96	1361	A-133537	asGfsaUfcAfcAfgCfuccGfgAfaGfcAfascsa	1421
AD-66765	A-133538	UfscsCfgGfaGfcUfGfUfgAfuCfuGfaGfgAfL96	1362	A-133539	usCfscUfcAfgAfuCfacaGfcUfcCfgGfasasg	1422
AD-66766	A-133540	GfsusGfgAfuGfaGfUfGfuUfgCfuUfcCfgAfL96	1363	A-133541	usCfsgGfaAfgCfaAfcaCfuCfaUfcAfcsasa	1423

Duplex Name	Sense Oligo Name	Modified Sense Sequence	SEQ ID NO	Antisense Oligo Name	Modified Antisense Sequence	SEQ ID NO:
AD-66767	A-133542	UfsasCfaCfaUfcAfUfGfuCfgUfcUfuCfaAfL96	1364	A-133543	usUfsgAfaGfaCfgAfcauGfaUfgUfgUfasusc	1424
AD-66768	A-133544	AfsasAfgAfuAfcAfCfAfuCfaUfgUfcGfuAfL96	1365	A-133545	usAfscGfaCfaUfgAfuguGfuAfuCfuUfusasu	1425
AD-66769	A-133546	GfsgsCfuAfuGfgCfUfCfcAfgCfaUfuCfgAfL96	1366	A-133547	usCfsgAfaUfgCfuGfgagCfcAfuAfgCfcsusg	1426
AD-66770	A-133548	AfscsAfcUfgAfcAfUfGfcCfcAfaGfaCfuAfL96	1367	A-133549	usAfsgUfcUfuGfgGfcauGfuCfaGfuGfusgsg	1427
AD-66771	A-133550	AfsgsUfgUfuGfcUfUfCfcGfgAfgCfuGfuAfL96	1368	A-133551	usAfscAfgCfuCfcGfgaaGfcAfaCfaCfuscsa	1428
AD-66772	A-133552	GfsasGfaCfcCfuUfUfGfcGfgGfgCfuGfaAfL96	1369	A-133553	usUfscAfgCfcCfcGfcaaAfgGfgUfcUfcsusg	1429
AD-66773	A-133554	AfscsUfgAfcAfuGfCfCfcAfaGfaCfuCfaAfL96	1370	A-133555	usUfsgAfgUfcUfuGfggcAfuGfuCfaGfusgsu	1430
AD-66774	A-133556	GfsasUfaCfaCfaUfCfAfuGfuCfgUfcUfuAfL96	1371	A-133557	usAfsaGfaCfgAfcAfugaUfgUfgUfaUfcsusu	1431
AD-66775	A-133558	AfsasGfcCfcAfcAfGfUfGfcUfuUfgGfcUfcAfL96	1372	A-133559	usGfsaGfcCfaUfaGfccuGfuGfgGfcUfusgsu	1432

Example 6 – Knockdown of IGF-1 expression with an IGF-1 siRNA decreases expression of IGF-1

A series of siRNAs targeting mouse IGF-1 were designed and tested for the ability to knockdown expression of IGF-1 mRNA in 6-8 week old C57Bl/6 female mice. Duplexes were selected for further optimization using chemical modifications. Analysis for IGF-1 knockdown in mice using the same assay identified the AD-68112 duplex (sense sequence gsasuacaCfaUfCfAfugucgucuuaL96 (SEQ ID NO:1433); antisense sequence usAfsagaCfgAfCfaugaUfgUfguaucsusu (SEQ ID NO: 1434), based on the sequence of the AD-66774 duplex, for use in further studies.

A single 3 mg/kg or10 mg/kg dose of AD-68112; or PBS control, was administered subcutaneously on day 0 to 6-8 week old C57Bl/6 female mice (n = 3 per group). On days 7, 14, and 21 the mice were sacrificed to assess knockdown of IGF-1 mRNA in liver by qPCR.

AD-68112 was found to be effective in decreasing expression of IGF-1 mRNA. The results are shown in the table below. Results are expressed as mRNA levels relative to control.

Dose	Day 7	Day 14	Day 21
3 mg/kg	7.4	17.8	33.3
10 mg/kg	3.4	7.4	12.7

In a separate study, a decrease in serum IGF-1 was observed in 6-8 week old C57Bl/6 female mice (n=3) response to treatment with AD-68112 in a single dose of 3 mg/kg. Specifically, AD-68112 decreased the serum IGF-1 protein level to about 17%, 70%, and 55% on days 7, 14, and 21, respectively.

Further, in a dose-response study, AD-68112 was demonstrated to be effective in knocking down the expression of IGF-1 mRNA in the liver in a dose- response manner. Specifically, C57Bl/6 female mice, 6-8 weeks of age (n = 3 per group) were administered a single 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg AD-68112 at; or a PBS control. IGF-1 serum protein level reduction was observed in a dose response manner.

Example 7 – Knockdown of IGFALS and IGF-1 expression with IGFALS and IGF-1 siRNAs alone and in combination

A series of siRNAs targeted each to mouse IGFALS (AD-66807, sense sequence, ascsagauGfaGfCfUfcagcgucuuuL96, SEQ ID NO: 1435; and antisense sequence asAfsagaCfgCfUfgagcUfcAfucugusgsu, SEQ ID NO:1436) and mouse IGF-1 (AD-68112) were tested for the ability to knockdown expression of IGFALS and IGF-1 mRNA and protein expression in 6-8 week old C57Bl/6 female mice, either alone or in combination.

Weekly 3 mg/kg doses of AD-66807 and AD-68112, either alone or in combination; or PBS control, were administered to 4 week old C57Bl/6 female mice (n = 8 per group) subcutaneously starting at day 0 for 8 weeks. On day 58 or 59, the mice were sacrificed to assess knockdown of

IGFALS and IGF-1 mRNA in liver by qPCR. Serum IGFALS levels were assayed by western blot and serum IGF-1 levels were assayed by ELISA.

AD-66807 and AD-68112 were found to be effective in decreasing expression of IGFALS and IGF-1 mRNA and protein. The results are shown in the tables below. RNA and protein levels are expressed as levels relative to control.

IGFALS and IGF-1 mRNA levels relative to control

Treatment	IGFALS	IGF-1
Control (PBS)	1.16	1.05
SiRNA-IGFALS (3 mg/kg)	0.11	1.10
SiRNA-IGF-1 (3 mg/kg)	0.70	0.03
SiRNA-ALS (3 mg/kg) +IGF-1 (3 mg/kg)	0.09	0.05

IGFALS and IGF-1 protein levels relative to control.

Treatment	IGFALS	IGF-1
Control (PBS)	1.0	1.0
SiRNA-IGFALS (3 mg/kg)	0.03	0.26
SiRNA-IGF-1 (3 mg/kg)	0.61	0.13
SiRNA-ALS (3 mg/kg) +IGF-1 (3 mg/kg)	0.01	0.05

A dose response study was performed. AD-66807 and AD-68112 were subcutaneously administered to 6-8 week old C57Bl/6 female mice (n = 3 per group) at 0.3 mg/kg, 1 mg/kg, 3 mg/kg, and 10 mg/kg, either alone or in combination; or PBS control starting at day 0. On day 14, the mice were sacrificed to assess knockdown of IGFALS and IGF-1 mRNA in liver by qPCR. Serum IGF-1 levels were assayed by ELISA.

AD-66807 and AD-68112 were found to be effective in decreasing expression of serum IGF-1 protein. The results are shown in the table below. Protein levels are expressed as levels relative to control.

Serum IGF-1 protein levels relative to control at Day 7.

Treatment	0 mg/kg	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg	10.0 mg/kg
SiRNA-IGFALS	100	0.81	0.54	0.37	0.31
SiRNA-IGF-1	100	1.13	0.83	0.52	0.49
SiRNA-IGFALS + SiRNA-IGF-1	100	0.61	0.34	0.14	0.06

Example 8 – Knockdown of IGFALS and IGF-1 expression with IGFALS and IGF-1 siRNAs alone and in combination in a transgenic mouse expressing bovine growth hormone

Similar knockdown of serum IGFALS and IGF-1 levels were observed in a transgenic mouse that constitutively expresses bovine growth hormone and recapitulates some of the features of acromegaly (Olsson et al, Am J Physiol Endocrinol Metab. 2003;285:E504-11). Specifically, AD-66807 and AD-68112 were demonstrated to decrease serum levels of IGF-1 protein. At least a trend in decreased weight gain was observed in male mice treated with either AD-66807 or AD-68112 alone, and in female mice treated with a combination of AD-66807 and AD-68112.

Example 9 -- IGFALS Transcripts, siRNA Design, and siRNA Screening

A set of siRNAs targeting the human IGFALS, "insulin like growth factor binding protein acid labile subunit" (human: NCBI refseqID NM_004970 (SEQ ID NO: 1); NCBI GeneID: 3483), as well as toxicology-species IGFALS orthologs (cynomolgus monkey: XM_005590898) were designed using custom R and Python scripts. The human NM_004970 REFSEQ mRNA, version 2, has a length of 2168 bases.

The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every potential 19mer siRNA from position 10 through the end was determined with a linear model derived the direct measure of mRNA knockdown from more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. Subsets of the IGFALS siRNAs were designed with perfect or near-perfect matches between human and cynomolgus monkey. For each strand of the siRNA, a custom Python script was used in a brute force search to measure the number and positions of mismatches between the siRNA and all potential alignments in the target species transcriptome. Extra weight was given to mismatches in the seed region, defined here as positions 2-9 of the antisense oligonucleotide, as well the cleavage site of the siRNA, defined here as positions 10-11 of the antisense oligonucleotide. The relative weight of the mismatches was 2.8; 1.2: 1 for seed mismatches, cleavage site, and other positions up through antisense position 19. Mismatches in the first position were ignored. A specificity score was calculated for each strand by summing the value of each weighted mismatch. Preference was given to siRNAs whose antisense score in human was >= 2.2 and predicted efficacy was >= 50% knockdown of the transcript.

In vitro Dual-Glo® screening

Cell culture and transfections

Cos7 cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in DMEM (ATCC) supplemented with 10% FBS, before being released from the plate by trypsinization. Human IGFALS (NM_004970 (SEQ ID NO:1) was cloned into the psicheck2 vector to generate the Dual-Glo® Luciferase construct. The Dual-luciferase plasmid was co-transfected with siRNA into 5000 cells using Lipofectamine RNAiMax (Invitrogen, Carlsbad CA. cat # 13778-150). For each well of a 384 well plate, 0.1µl of Lipofectamine was added to 5ng of plasmid vector and siRNA in 15µl of Opti-MEM and allowed to complex at room temperature for 15 minutes. The

mixture was then added to the cells resuspended in 35ul of fresh complete media. Cells were incubated for 48 hours before luciferase was measured. Screens were performed at 10nM and 0.1nM final duplex concentration.

Dual-Glo® Luciferase assay

Forty-eight hours after the siRNAs were transfected, Firefly (transfection control) and Renilla (fused to IGFALS target sequence in 3' UTR) luciferase were measured. First, media was removed from cells. Then Firefly luciferase activity was measured by adding 20ul of Dual-Glo® Luciferase Reagent mixed with 20ul of complete media to each well. The mixture was incubated at room temperature for 30 minutes before luminescense (500nm) was measured on a Spectramax (Molecular Devices) to detect the Firefly luciferase signal. Renilla luciferase activity was measured by adding 20ul of room temperature of Dual-Glo® Stop & Glo® Reagent to each well and the plates were incubated for 20 minutes before luminescence was again measured to determine the Renilla luciferase signal. The Dual-Glo® Stop & Glo® Reagent quenched the firefl y luciferase signal and sustained luminescence for the Renilla luciferase reaction. siRNA activity was determined by normalizing the Renilla (IGFALS) signal to the Firefly (control) signal within each well. The magnitude of siRNA activity was then assessed relative to cells that were transfected with the same vector but were not treated with siRNA or were treated with a non-targeting siRNA. All transfections were done in quadruplicates.

Table 12. Unmodified Sense and Antisense Strand Sequences of IGFALS dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 004970.2	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 004970.2	SEQ ID NO
AD-76171	A-152158	CAAUUAAAGGCAAAGGCAAUA	2058-2078	1437	A-152159	UAUUGCCUUUGCCUUUAAUUGAU	2056-2078	1484
AD-76172	A-152160	ACACCUACAACAACAUCACCA	1808-1828	1438	A-152161	UGGUGAUGUUGUUGUAGGUGUAC	1806-1828	1485
AD-76173	A-152162	UACACCUACAACAACAUCACA	1807-1827	1439	A-152163	UGUGAUGUUGUUGUAGGUGUACG	1805-1827	1486
AD-76174	A-152164	CAUCAAGGCAAACGUGUUCGA	777-797	1440	A-152165	UCGAACACGUUUGCCUUGAUGGC	775-797	1487
AD-76175	A-152166	CACCUACAACAACAUCACCUA	1809-1829	1441	A-152167	UAGGUGAUGUUGUUGUAGGUGUA	1807-1829	1488
AD-76176	A-152168	CGUACACCUACAACAACAUCA	1805-1825	1442	A-152169	UGAUGUUGUUGUAGGUGUACGCG	1803-1825	1489
AD-76177	A-152170	GUACACCUACAACAACAUCAA	1806-1826	1443	A-152171	UUGAUGUUGUUGUAGGUGUACGC	1804-1826	1490
AD-76178	A-152172	GACUCUUCCUCAAGGACAACA	1322-1342	1444	A-152173	UGUUGUCCUUGAGGAAGAGUCGG	1320-1342	1491
AD-76179	A-152174	AGCCUUCCAGAACCUCUCCAA	357-377	1445	A-152175	UUGGAGAGGUUCUGGAAGGCUGC	355-377	1492
AD-76180	A-152176	UCACGCUAGACCACAACCAGA	1109-1129	1446	A-152177	UCUGGUUGUGGUCUAGCGUGAGC	1107-1129	1493
AD-76181	A-152178	UGGACGUCUCGCACAACCGCA	1541-1561	1447	A-152179	UGCGGUUGUGCGAGACGUCCAGC	1539-1561	1494
AD-76182	A-152180	ACCUGGACCGCAACCUCAUCA	824-844	1448	A-152181	UGAUGAGGUUGCGGUCCAGGUAG	822-844	1495
AD-76183	A-152182	UGGACCUGUCCCACAACCGCA	893-913	1449	A-152183	UGCGGUUGUGGGACAGGUCCAGC	891-913	1496
AD-76184	A-152184	UGCGGCUGUCCCACAACGCCA	965-985	1450	A-152185	UGGCGUUGUGGGACAGCCGCAGC	963-985	1497
AD-76185	A-152186	CGCUCGGCCUCAGCAACAACA	530-550	1451	A-152187	UGUUGUUGCUGAGGCCGAGCGAG	528-550	1498
AD-76186	A-152188	CCUCAGGAACAACUCACUGCA	1617-1637	1452	A-152189	UGCAGUGAGUUGUUCCUGAGGCU	1615-1637	1499
AD-76187	A-152190	CUGCGGACCUUCACGCCGCAA	1633-1653	1453	A-152191	UUGCGGCGUGAAGGUCCGCAGUG	1631-1653	1500
AD-76188	A-152192	CCUCUCUGGGAACUGUCUCCA	1185-1205	1454	A-152193	UGGAGACAGUUCCCAGAGAGGUU	1183-1205	1501
AD-76189	A-152194	GCUCUCCCGCAACCGCCUGGA	1473-1493	1455	A-152195	UCCAGGCGGUUGCGGGAGAGCAG	1471-1493	1502
AD-76190	A-152196	CGUCUCGCACAACCGCCUGGA	1545-1565	1456	A-152197	UCCAGGCGGUUGUGCGAGACGUC	1543-1565	1503
AD-76191	A-152198	CACGCUAGACCACAACCAGCA	1110-1130	1457	A-152199	UGCUGGUUGUGGUCUAGCGUGAG	1108-1130	1504
AD-76192	A-152200	UGCUCUCCCGCAACCGCCUGA	1472-1492	1458	A-152201	UCAGGCGGUUGCGGGAGAGCAGC	1470-1492	1505
AD-76193	A-152202	ACCUCAGCCUCAGGAACAACA	1610-1630	1459	A-152203	UGUUGUUCCUGAGGCUGAGGUAG	1608-1630	1506
AD-76194	A-152204	CACCUUCAAGGACCUGCACUA	1005-1025	1460	A-152205	UAGUGCAGGUCCUUGAAGGUGCG	1003-1025	1507

AD-76195	A-152206	GGCCUCGUGGGCAUUGAGGAA	1342-1362	1461	A-152207	UUCCUCAAUGCCCACGAGGCCGU	1340-1362	1508
AD-76196	A-152208	ACCUUCAAGGACCUGCACUUA	1006-1026	1462	A-152209	UAAGUGCAGGUCCUUGAAGGUGC	1004-1026	1509
AD-76197	A-152210	GGCCUUCUGGCUGGAGACGUCUA	1530-1550	1463	A-152211	UAGACGUCCAGCCAGAAGGCCCG	1528-1550	1510
AD-76198	A-152212	GGCAUUGAGGAGCAGAGCCUA	1351-1371	1464	A-152213	UAGGCUCUGCUCCUCAAUGCCCA	1349-1371	1511
AD-76199	A-152214	GCCUUCCAGAACCUCUCCAGA	358-378	1465	A-152215	UCUGGAGAGGUUCUGGAAGGCUG	356-378	1512
AD-76200	A-152216	GCGGUCAUGAACCUCUCUGGA	1174-1194	1466	A-152217	UCCAGAGAGGUUCAUGACCGCCA	1172-1194	1513
AD-76201	A-152218	AGGCUGGAGGACGGGCUCUUA	559-579	1467	A-152219	UAAGAGCCCGUCCUCCAGCCUGC	557-579	1514
AD-76202	A-152220	CUCUACCUGGACCGCAACCUA	820-840	1468	A-152221	UAGGUUGCGGUCCAGGUAGAGUU	818-840	1515
AD-76203	A-152222	GCGGAUGAGCUCAGCGUCUUA	238-258	1469	A-152223	UAAGACGCUGAGGCUCAUCCGCGU	236-258	1516
AD-76204	A-152224	CCGUCUGAGCAGGCUGGAGGA	549-569	1470	A-152225	UCCUCCAGCCUGCUCAGACGGUU	547-569	1517
AD-76205	A-152226	GGUCAUGAACCUCUCUGGGAA	1176-1196	1471	A-152227	UUCCCAGAGAGGUUCAUGACCGC	1174-1196	1518
AD-76206	A-152228	CUGCAGCUGGGCCACAACCGA	1036-1056	1472	A-152229	UCGGUUGUGGCCCAGCUGCAGCU	1034-1056	1519
AD-76207	A-152230	CGGGAGCUGGACCUGAGCAGA	742-762	1473	A-152231	UCUGCUCAGGUCCAGCUCCCGGA	740-762	1520
AD-76208	A-152232	CCGCCUGUGUCUGCAGCUACA	209-229	1474	A-152233	UGUAGCUGCAGACACAGGCGGCC	207-229	1521
AD-76209	A-152234	UCUUCUGCAGCUCCCAGGAACA	254-274	1475	A-152235	UGUUCCUGGAGCUGCAGAAGACG	252-274	1522
AD-76210	A-152236	CUGGCUGAGCGCAGCUUUGAA	1066-1086	1476	A-152237	UUCAAAGCUGCGCUCAGCCAGCU	1064-1086	1523
AD-76211	A-152238	CUCGACCUGACCUCCAACCAA	1396-1416	1477	A-152239	UUGGUUGGAGGUCAGGUCGAGCU	1394-1416	1524
AD-76212	A-152240	CUCAACCUCCGGCUGGAAUAGA	604-624	1478	A-152241	UCUAUUCCAGCCGAGGUUGAGGU	602-624	1525
AD-76213	A-152242	GCCUAGAGAACCUGUGCCACA	440-460	1479	A-152243	UGUGGCACAGGUUCUCUAGGCCC	438-460	1526
AD-76214	A-152244	CAGCUUGAGGUGCUCACGCUA	1096-1116	1480	A-152245	UAGCGUGAGCACCUCAAGCUGCC	1094-1116	1527
AD-76215	A-152246	CAGGUCCUCAGUGUCCUCAGA	1961-1981	1481	A-152247	UCUGAGGACACUGAGGACCUGUC	1959-1981	1528
AD-76216	A-152248	GCUGCGAUGGCUGGACCUGUA	882-902	1482	A-152249	UACAGGUCCAGCCAUCGCAGCGC	880-902	1529
AD-76217	A-152250	GGCAAGCUGGAGUACCUGCUA	1453-1473	1483	A-152251	UAGCAGGUACUCCAGCUUGCCCA	1451-1473	1530

Table 13. IGFALS in vitro 10nM and 0.1nM screen

Duplex Name	10nM AVG	10nM STD	0.1nM AVG	0.1nM STD	Position in NM_ 004970.2
AD-76171	29.9	0.1	55.8	5.4	2058-2078
AD-76172	42.7	2.3	101.9	7.8	1808-1828
AD-76173	32.8	3.1	81.2	7.1	1807-1827
AD-76174	45.3	10.5	86.2	9.7	777-797
AD-76175	42.8	7.6	97.3	8.7	1809-1829
AD-76176	69	5.8	93.9	11.3	1805-1825
AD-76177	53.6	11	99.2	15.8	1806-1826
AD-76178	72.6	4.9	92.6	6.9	1322-1342
AD-76179	80.1	8.2	113.3	8	357-377
AD-76180	132	13.9	124.2	16.8	1109-1129
AD-76181	67.1	5.9	99.1	0.3	1541-1561
AD-76182	110.4	17.9	102	5.1	824-844
AD-76183	69.8	9.9	100.3	6.9	893-913
AD-76184	62	6.3	99.4	7.1	965-985
AD-76185	119.5	33.1	92.8	4.4	530-550
AD-76186	52.4	4.6	94.7	2.7	1617-1637
AD-76187	116.7	6.4	117.7	9.4	1633-1653
AD-76188	60.4	5.8	106.2	4.8	1185-1205
AD-76189	89.9	2.1	102.9	8.4	1473-1493
AD-76190	83.5	3.5	104.5	6.5	1545-1565
AD-76191	80.9	3.3	96.4	7.2	1110-1130
AD-76192	93.9	3.9	103	5.4	1472-1492
AD-76193	99.3	3.5	95.1	10.2	1610-1630
AD-76194	192.1	4.9	118	12.1	1005-1025
AD-76195	86.1	3.3	106.7	8.4	1342-1362
AD-76196	184	23.4	114.8	6.7	1006-1026
AD-76197	55.3	4.4	111.4	14.2	1530-1550
AD-76198	66.7	3.6	97.1	13.1	1351-1371
AD-76199	54.5	2	91.8	4.2	358-378
AD-76200	63.9	10.1	88	10.6	1174-1194
AD-76201	150.6	4.6	113.6	18.3	559-579
AD-76202	64.7	1.4	95.7	13.7	820-840
AD-76203	41.3	1.7	93	2.1	238-258
AD-76204	67.5	6.9	101.3	3.6	549-569
AD-76205	73.1	9.5	87.6	7.8	1176-1196
AD-76206	86.4	3.7	107.7	17	1036-1056
AD-76207	131.4	22.4	99.7	10.5	742-762

Duplex Name	10nM AVG	10nM STD	0.1nM AVG	0.1nM STD	Position in NM_ 004970.2
AD-76208	46.5	12	103	13.2	209-229
AD-76209	43.8	5.2	98.1	13.9	254-274
AD-76210	41.6	7.3	94.4	6	1066-1086
AD-76211	152.3	9.4	125.1	3.4	1396-1416
AD-76212	59.2	12.7	82.3	23.4	604-624
AD-76213	71.1	4.9	94.9	11.6	440-460
AD-76214	109.8	5.9	102.8	8.4	1096-1116
AD-76215	70.2	7.5	103.2	25.4	1961-1981
AD-76216	62.8	9.5	107.7	15.1	882-902
AD-76217	68.8	1.7	94.5	2.4	1453-1473
Mock	117.3	16.3	106.9	9.5	

Table 14. Modified Sense and Antisense Strand Sequences of IGFALS dsRNAs

Duplex Name	Sense Oligo Name	Sense OligoSequence	SEQ ID NO	Antisense Oligo Name	Antisense Oligo Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-76171	A-152158	csasauuaAfaGfGfCfaaaggcaauaL96	1531	A-152159	VPusAfsuugCfcUfUfugccUfuUfaauugsasu	1578	AUCAAUUAAAGGCAAAGGCAAUC	1625
AD-76172	A-152160	ascsaccuAfcAfAfCfaacaucaccaL96	1532	A-152161	VPusGfsgugAfuGfUfuguuGfuAfggugusasc	1579	GUACACCUACAACAACAUCACCU	1626
AD-76173	A-152162	usascaccUfaCfAfAfcaacaucacaL96	1533	A-152163	VPusGfsugaUfgUfUfguugUfaGfguguascsg	1580	CGUACACCUACAACAACAUCACC	1627
AD-76174	A-152164	csasucaaGfgCfAfAfacguguucgaL96	1534	A-152165	VPusCfsgaaCfaCfGfuuugCfcUfugaugsgsc	1581	GCCAUCAAGGCAAACGUGUUCGU	1628
AD-76175	A-152166	csasccuaCfaAfCfAfacaucaccuaL96	1535	A-152167	VPusAfsgguGfaUfGfuuguUfgUfaggugsusa	1582	UACACCUACAACAACAUCACCUG	1629
AD-76176	A-152168	csgsuacaCfcUfAfCfaacaacaucaL96	1536	A-152169	VPusGfsaugUfuGfUfuguaGfgUfguacgscsg	1583	CGCGUACACCUACAACAACAUCA	1630
AD-76177	A-152170	gsusacacCfuAfCfAfacaacaucaaL96	1537	A-152171	VPusUfsgauGfuUfGfuuguAfgGfuguacsgsc	1584	GCGUACACCUACAACAACAUCAC	1631
AD-76178	A-152172	gsascucuUfcCfUfCfaaggacaacaL96	1538	A-152173	VPusGfsuugUfcCfUfugagGfaAfgagucsgsg	1585	CCGACUCUUCCUCAAGGACAACG	1632
AD-76179	A-152174	asgsccuuCfcAfGfAfaccucuccaaL96	1539	A-152175	VPusUfsggaGfaGfGfuucuGfgAfaggcusgsc	1586	GCAGCCUUCCAGAACCUCUCCAG	1633
AD-76180	A-152176	uscsacgcUfaGfAfCfcacaaccagaL96	1540	A-152177	VPusCfsuggUfuGfUfggucUfaGfcgugasgsc	1587	GCUCACGCUAGACCACAACCAGC	1634
AD-76181	A-152178	usgsgacgUfcUfCfGfcacaaccgcaL96	1541	A-152179	VPusGfscggUfuGfUfgcgaGfaCfguccasgsc	1588	GCUGGACGUCUCGCACAACCGCC	1635
AD-76182	A-152180	ascscuggAfcCfGfCfaaccucaucaL96	1542	A-152181	VPusGfsaugAfgGfUfugcgGfuCfcaggusasg	1589	CUACCUGGACCGCAACCUCAUCG	1636
AD-76183	A-152182	usgsgaccUfgUfCfCfcacaaccgcaL96	1543	A-152183	VPusGfscggUfuGfUfgggaCfaGfguccasgsc	1590	GCUGGACCUGUCCCACAACCGCG	1637
AD-76184	A-152184	usgscggcUfgUfCfCfcacaacgccaL96	1544	A-152185	VPusGfsgcgUfuGfUfgggaCfaGfccgcasgsc	1591	GCUGCGGCUGUCCCACAACGCCA	1638
AD-76185	A-152186	csgscucgGfcCfUfCfagcaacaacaL96	1545	A-152187	VPusGfsuugUfuGfCfugagGfcCfgagcgsasg	1592	CUCGCUCGGCCUCAGCAACAACC	1639
AD-76186	A-152188	cscsucagGfaAfCfAfacucacugcaL96	1546	A-152189	VPusGfscagUfgAfGfuuguUfcCfugaggscsu	1593	AGCCUCAGGAACAACUCACUGCG	1640
AD-76187	A-152190	csusgcggAfcCfUfUfcacgccgcaaL96	1547	A-152191	VPusUfsgcgGfcGfUfgaagGfuCfcgcagsusg	1594	CACUGCGGACCUUCACGCCGCAG	1641
AD-76188	A-152192	cscsucucUfgGfGfAfacugucuccaL96	1548	A-152193	VPusGfsgagAfcAfGfuuccCfaGfagaggsusu	1595	AACCUCUCUGGGAACUGUCUCCG	1642
AD-76189	A-152194	gscsucucCfcGfCfAfaccgccuggaL96	1549	A-152195	VPusCfscagGfcGfGfuugcGfgGfagagcsasg	1596	CUGCUCUCCCGCAACCGCCUGGC	1643
AD-76190	A-152196	csgsucucGfcAfCfAfaccgccuggaL96	1550	A-152197	VPusCfscagGfcGfGfuuguGfcGfagacgsusc	1597	GACGUCUCGCACAACCGCCUGGA	1644
AD-76191	A-152198	csascgcuAfgAfCfCfacaaccagcaL96	1551	A-152199	VPusGfscugGfuUfGfugguCfuAfgcgugsasg	1598	CUCACGCUAGACCACAACCAGCU	1645
AD-76192	A-152200	usgscucuCfcCfGfCfaaccgccugaL96	1552	A-152201	VPusCfsaggCfgGfUfugcgGfgAfgagcasgsc	1599	GCUGCUCUCCCGCAACCGCCUGG	1646
AD-76193	A-152202	ascscucaGfcCfUfCfaggaacaacaL96	1553	A-152203	VPusGfsuugUfuCfCfugagGfcUfgaggusasg	1600	CUACCUCAGCCUCAGGAACAACU	1647
AD-76194	A-152204	csascuuCfaAfGfGfaccugcacuaL96	1554	A-152205	VPusAfsgugCfaGfGfuccuUfgAfaggugscsg	1601	CGCACCUUCAAGGACCUGCACUU	1648
AD-76195	A-152206	gsgscucGfuGfGfGfcauugaggaaL96	1555	A-152207	VPusUfsccuCfaAfUfgcccAfcGfaggccsgsu	1602	ACGGCCUCGUGGGCAUUGAGGAG	1649

Duplex Name	Sense Oligo Name	Sense OligoSequence	SEQ ID NO	Antisense Oligo Name	Antisense Oligo Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-76196	A-152208	ascscuucAfaGfGfAfccugcacuuaL96	1556	A-152209	VPusAfsaguGfcAfGfguccUfuGfaaggusgsc	1603	GCACCUUCAAGGACCUGCACUUC	1650
AD-76197	A-152210	gsgsccuuChuGfGfCfuggacgucuaL96	1557	A-152211	VPusAfsgacGfuCfCfagccAfgAfaggccscsg	1604	CGGGCCUUCUGGCUGGACGUCUC	1651
AD-76198	A-152212	gsgscauuGfaGfGfAfgcagagccuaL96	1558	A-152213	VPusAfsggcUfcUfGfcuccUfcAfaugccscsa	1605	UGGGCAUUGAGGAGCAGAGCCUG	1652
AD-76199	A-152214	gscscuucCfaGfAfAfccucccagaL96	1559	A-152215	VPusCfsuggAfgAfGfguucUfgGfaaggcsusg	1606	CAGCCUUCCAGAACCUCUCCAGC	1653
AD-76200	A-152216	gscsggucAfuGfAfAfccucuggaaL96	1560	A-152217	VPusCfscagAfgAfGfguucAfuGfaccgcscsa	1607	UGGCGGUCAUGAACCUCUCUGGG	1654
AD-76201	A-152218	asgsgcugGfaGfGfAfcgggcucuuaL96	1561	A-152219	VPusAfsagaGfcCfCfguccUfcCfagccusgsc	1608	GCAGGCUGGGAGGACGGGCUCUUC	1655
AD-76202	A-152220	csuscuacCfuGfGfAfccgcaaccuaL96	1562	A-152221	VPusAfsgguUfgCfGfguccAfgGfuagagsusu	1609	AACUCUACCUGGACCGCAACCUC	1656
AD-76203	A-152222	gscsggauGfaGfCfUfcagcgucuuaL96	1563	A-152223	VPusAfsagaCfgCfUfgagcUfcAfuccgcsgsu	1610	ACGCGGAUGAGCUCAGCGUCUUC	1657
AD-76204	A-152224	cscsgucuGfaGfCfAfggcuggaggaL96	1564	A-152225	VPusCfscucCfaGfCfcugcUfcAfgacggsusu	1611	AACCGUCUGAGCAGGCUGGAGGA	1658
AD-76205	A-152226	gsgsucauGfaAfCfCfcucuggggaaL96	1565	A-152227	VPusUfscccAfgAfGfagguUfcAfugaccsgsc	1612	GCGGUCAUGAACCUCUCUGGGAA	1659
AD-76206	A-152228	csusgcagCfuGfGfGfccacaaccgaL96	1566	A-152229	VPusCfsgguUfgUfGfgcccAfgCfugcagscsu	1613	AGCUGCAGCUGGGCCACAACCGC	1660
AD-76207	A-152230	csgsggagCfuGfGfAfAfccugagcagaL96	1567	A-152231	VPusCfsugcUfcAfGfguccAfgCfucccgsgsa	1614	UCCGGGAGCUGGACCUGAGCAGG	1661
AD-76208	A-152232	cscsgccuGfuGfUfCfUfcugcagcuacaL96	1568	A-152233	VPusGfsuagCfuGfCfagacAfcAfggcggscsc	1615	GGCCGCCUGUGUCUGCAGCUACG	1662
AD-76209	A-152234	uscsuucuGfcAfGfCfUfccaggaacaL96	1569	A-152235	VPusGfsuucCfuGfGfagcuGfcAfgaagascsg	1616	CGUCUUCUGCAGCUCCAGGAACC	1663
AD-76210	A-152236	csusggcuGfaGfCfCfGfcagcuuugaaL96	1570	A-152237	VPusUfscaaAfgCfUfgcgcUfcAfgccagscsu	1617	AGCUGGCUGAGCGCAGCUUUGAG	1664
AD-76211	A-152238	csuscgacCfuGfAfAfCfcuccaaccaaL96	1571	A-152239	VPusUfsgguUfgGfAfggucAfgGfucgagscsu	1618	AGCUCGACCUGACCUCCCAACCAG	1665
AD-76212	A-152240	csuscaacCfuCfGfGfcuggaauaagaL96	1572	A-152241	VPusCfsuauUfcCfAfgcccgAfgGfuugagsgsu	1619	ACCUCAACCUCCGGCUGGAAUAGC	1666
AD-76213	A-152242	gscscuagAfgAfAfCfCfcugugccacaL96	1573	A-152243	VPusGfsuggCfaCfAfgguuCfuCfuaggcscsc	1620	GGGCCUAGAGAACCUGUGCCACC	1667
AD-76214	A-152244	csasgcuuGfaGfGfUfUfgcucacgcuaL96	1574	A-152245	VPusAfsgcgUfgAfGfcaccUfcAfagcugscsc	1621	GGCAGCUUGAGGUGCUCACGCUA	1668
AD-76215	A-152246	csasgguCfuCfAfGfuguccucagaL96	1575	A-152247	VPusCfsugaGfgAfCfacugAfgGfaccugsusc	1622	GACAGGUCCUCAGUGUCCUCAGG	1669
AD-76216	A-152248	gscsugcgAfuGfGfCfUfuggaccuguaL96	1576	A-152249	VPusAfscagGfuCfCfagccAfuCfgcagcsgsc	1623	GCGCUGCGAUGGCUGGACCUGUC	1670
AD-76217	A-152250	gsgscaagCfuGfGfGfAfguaccugcuaL96	1577	A-152251	VPusAfsgcaGfgUfAfcuccAfgCfuugccscsa	1624	UGGGCAAGCUGGAGUACCUGCUG	1671

Example 10 – IGF-1 Transcripts, siRNA Design, and siRNA Screening

Bioinformatics

A set of siRNAs targeting the human IGF1, ("insulin like growth factor 1", NCBI refseqID: NM_000618; NCBI GeneID: 3479 (SEQ ID NO:13), as well as toxicology-species IGF1 orthologs (cynomolgus monkey: XM_005572039) were designed using custom R and Python scripts. The human NM_000618 REFSEQ mRNA, version 3, has a length 7321 of bases.

The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every potential 19mer siRNA from position 10 through the end was determined with a linear model derived the direct measure of mRNA knockdown from more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. Subsets of the IGF1 siRNAs were designed with perfect or near-perfect matches between human and cynomolgus monkey. For each strand of the siRNA, a custom Python script was used in a brute force search to measure the number and positions of mismatches between the siRNA and all potential alignments in the target species transcriptome. Extra weight was given to mismatches in the seed region, defined here as positions 2-9 of the antisense oligonucleotide, as well the cleavage site of the siRNA, defined here as positions 10-11 of the antisense oligonucleotide. The relative weight of the mismatches was 2.8; 1.2: 1 for seed mismatches, cleavage site, and other positions up through antisense position 19. Mismatches in the first position were ignored. A specificity score was calculated for each strand by summing the value of each weighted mismatch. Preference was given to siRNAs whose antisense score in human was >= 2.2 and predicted efficacy was >= 50% knockdown of the transcript.

In vitro Dual-Glo® screening

Cell culture and transfections

Cos7 cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in DMEM (ATCC) supplemented with 10% FBS, before being released from the plate by trypsinization. Three human IGF-1 Dual-Glo® Luciferase constructs were generated using the psiCHECK2 vector. Construct one contained sequence based on NM_001111285 (SEQ ID NO:1672), while constructs two and three contained sequence based on NM_000618 (SEQ ID NOs: 1673 and 1674). Dual-luciferase plasmids were co-transfected with siRNA into 5000 cells using Lipofectamine RNAiMax (Invitrogen, Carlsbad CA. cat # 13778-150). For each well of a 384 well plate, 0.1µl of Lipofectamine was added to 5ng of plasmid vector and siRNA in 15µl of Opti-MEM and allowed to complex at room temperature for 15 minutes. The mixture was then added to the cells resuspended in 35ul of fresh complete media. Cells were incubated for 48 hours before luciferase was measured. Screen was performed at 10nM and 0.1nM final duplex concentration.

Dual-Glo® Luciferase assay

48 hours after the siRNAs were transfected, Firefly (transfection control) and Renilla (fused to IGF1 target sequence in 3' UTR) luciferase were measured. First, media was removed from cells. Then Firefly luciferase activity was measured by adding 20ul of Dual-Glo® Luciferase Reagent

mixed with 20ul of complete media to each well. The mixture was incubated at room temperature for 30 minutes before luminescense (500nm) was measured on a Spectramax (Molecular Devices) to detect the Firefly luciferase signal. Renilla luciferase activity was measured by adding 20ul of room temperature of Dual-Glo® Stop & Glo® Reagent to each well and the plates were incubated for 20 minutes before luminescence was again measured to determine the Renilla luciferase signal. The Dual-Glo® Stop & Glo® Reagent quenched the firefl y luciferase signal and sustained luminescence for the Renilla luciferase reaction. siRNA activity was determined by normalizing the Renilla (IGF1) signal to the Firefly (control) signal within each well. The magnitude of siRNA activity was then assessed relative to cells that were transfected with the same vector but were not treated with siRNA or were treated with a non-targeting siRNA. All transfections were done in quadruplicates.

Table 15. Unmodified Sense and Antisense Strand Sequences of IGF-1dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 000618.3	SEQ ID NO
AD-75740	A-151647	AAUGUAACUAAUUGAAUCAUA	7181-7201	1675	A-151648	UAUGAUUCAAUUAGUUACAUUUU	7179-7201	1768
AD-75741	A-151649	UAAGAAAGUACUUGACUAAAA	4362-4382	1676	A-151650	UUUUAGUCAAGUACUUUCUUAAA	4360-4382	1769
AD-75747	A-151661	CUUUAUAAUUCUUGAAUGAGA	3765-3785	1677	A-151662	UCUCAUUCAAGAAUUAUAAAGCA	3763-3785	1770
AD-75748	A-151663	AGAUAGAAAUGUAUGUUUGAA	5223-5243	1678	A-151664	UUCAAACAUACAUUUCUAUCUAG	5221-5243	1771
AD-75749	A-151665	UUCCACAAUCCUUAGAAUCUA	6512-6532	1679	A-151666	UAGAUUCUAAGGAUUGUGGAAGG	6510-6532	1772
AD-75750	A-151667	UAUCAAAACCUUUCAAAUAUA	6831-6851	1680	A-151668	UAUAUUUGAAAGGUUUUGAUAUU	6829-6851	1773
AD-75751	A-151669	ACAAGUAAACAUUCCAACAUA	824-844	1681	A-151670	UAUGUUGGAAUGUUUACUUGUGU	822-844	1774
AD-75755	A-151677	ACAUAGAAAGUUUCUUCAACA	1410-1430	1682	A-151678	UGUUGAAGAAACUUUCUAUGUUU	1408-1430	1775
AD-75757	A-151681	CAGUCAACAAGUAUUUUAACA	3122-3142	1683	A-151682	UGUUAAAAUACUUGUUGACUGAA	3120-3142	1776
AD-75759	A-151685	CUCAAGCUGUCUACUUACAUA	6769-6789	1684	A-151686	UAUGUAAGUAGACAGCUUGAGGU	6767-6789	1777
AD-75760	A-151687	GAAUUGUUUCCUUAUUUGCAA	1085-1105	1685	A-151688	UUGCAAAUAAGGAAACAAUUCAU	1083-1105	1778
AD-75761	A-151689	AUCUGUCUUAGUUGAAAAGCA	7071-7091	1686	A-151690	UGCUUUUCAACUAAGACAGAUGU	7069-7091	1779
AD-75765	A-151697	AAUUAGCAAUAUAUUAUCCAA	4632-4652	1687	A-151698	UUGGAUAAUAUAUUGCUAAUUUU	4630-4652	1780
AD-75766	A-151699	AAUUGAAUCAUUAUCUUACAA	7190-7210	1688	A-151700	UUGUAAGAUAAUGAUUCAAUUAG	7188-7210	1781
AD-75769	A-151705	UUUAUCAAUAAUGUUCUAUAA	908-928	1689	A-151706	UUAUAGAACAUUAUUGAUAAAAG	906-928	1782
AD-75772	A-151711	GUAAAAGAAACUAUACAUCAA	3213-3233	1690	A-151712	UUGAUGUAUAGUUUCUUUUACAU	3211-3233	1783
AD-75774	A-151715	AAUGAGGAAUAAUAAGUUAAA	5173-5193	1691	A-151716	UUUAACUUAUUAUUCCUCAUUCU	5171-5193	1784
AD-75776	A-151719	CUCUGUGAAUGUGUUUUAUCA	2737-2757	1692	A-151720	UGAUAAAACACAUUCACAGAGAG	2735-2757	1785
AD-75778	A-151723	GUUCCUUCAAAUGAUGAGUUA	1438-1458	1693	A-151724	UAACUCAUCAUUUGAAGGAACUC	1436-1458	1786
AD-75779	A-151725	CAGGAUAAAGAUAUCAAUUUA	5722-5742	1694	A-151726	UAAAUUGAUAUCUUUAUCCUGUA	5720-5742	1787
AD-75787	A-151741	CAUGUCCUCCUCGCAUCUCUA	297-317	1695	A-151742	UAGAGAUGCGAGGAGGACAUGGU	295-317	1788
AD-75787	A-151741	CAUGUCCUCCUCGCAUCUCUA	297-317	1696	A-151742	UAGAGAUGCGAGGAGGACAUGGU	295-317	1789
AD-75788	A-151743	UUCAACAAGCCCACAGGGUAA	436-456	1697	A-151744	UUACCCUGUGGGCUUGUUGAAAU	434-456	1790

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_000618.3	SEQ ID NO
AD-75788	A-151743	UUCAACAAGCCCACAGGGUAA	436-456	1698	A-151744	UUACCCUGUGGGCUUGUUGAAAU	434-456	1791
AD-75789	A-151745	UCCUCGCAUCUCUUCUACCUA	304-324	1699	A-151746	UAGGUAGAAGAGAUGCGAGGAGG	302-324	1792
AD-75789	A-151745	UCCUCGCAUCUCUUCUACCUA	304-324	1700	A-151746	UAGGUAGAAGAGAUGCGAGGAGG	302-324	1793
AD-75790	A-151747	GGGGCUUUUAUUUCAACAAGA	425-445	1701	A-151748	UCUUGUUGAAAUAAAAAGCCCCUG	423-445	1794
AD-75790	A-151747	GGGGCUUUUAUUUCAACAAGA	425-445	1702	A-151748	UCUUGUUGAAAUAAAAAGCCCCUG	423-445	1795
AD-75791	A-151749	GAUGCUCUUCAGUUCGUGUGA	397-417	1703	A-151750	UCACACGAACUGAAGAGCAUCCA	395-417	1796
AD-75791	A-151749	GAUGCUCUUCAGUUCGUGUGA	397-417	1704	A-151750	UCACACGAACUGAAGAGCAUCCA	395-417	1797
AD-75792	A-151751	UGGAUGCUCUUCAGUUCGUGA	395-415	1705	A-151752	UCACGAACUGAAGAGCAUCCACC	393-415	1798
AD-75792	A-151751	UGGAUGCUCUUCAGUUCGUGA	395-415	1706	A-151752	UCACGAACUGAAGAGCAUCCACC	393-415	1799
AD-75793	A-151753	GGUGGAUGCUCUUCAGUUCGA	393-413	1707	A-151754	UCGAACUGAAGAGCAUCCACCAG	391-413	1800
AD-75793	A-151753	GGUGGAUGCUCUUCAGUUCGA	393-413	1708	A-151754	UCGAACUGAAGAGCAUCCACCAG	391-413	1801
AD-75794	A-151755	GCUGGUGGAUGCUCUUCAGUA	390-410	1709	A-151756	UACUGAAGAGCAUCCACCAGCUC	388-410	1802
AD-75794	A-151755	GCUGGUGGAUGCUCUUCAGUA	390-410	1710	A-151756	UACUGAAGAGCAUCCACCAGCUC	388-410	1803
AD-75795	A-151757	CUGGUGGAUGCUCUUCAGUUA	391-411	1711	A-151758	UAACUGAAGAGCAUCCACCAGCU	389-411	1804
AD-75795	A-151757	CUGGUGGAUGCUCUUCAGUUA	391-411	1712	A-151758	UAACUGAAGAGCAUCCACCAGCU	389-411	1805
AD-77120	A-154752	CCAAAAUGCACUGAUGUAAAA	6586-6606	1713	A-154753	UUUUACAUCAGUGCAUUUUGGGC	6584-6606	1806
AD-77121	A-154754	UCCAGUUGCACUAAAAUUCCUA	1009-1029	1714	A-154755	UAGGAAUUUAGUGCAACUGGAUC	1007-1029	1807
AD-77122	A-154756	CAUUCUUCAACUAUCUUUGAA	6325-6345	1715	A-154757	UUCAAAGAUAGUUGAAGAAUGAG	6323-6345	1808
AD-77123	A-154758	ACAUUCCAACAUUGUCUUUAA	832-852	1716	A-154759	UUAAAGACAAUGUUGGAAUGUUU	830-852	1809
AD-77124	A-154760	GGCUUAGAAUAAAAGAUGUAA	4280-4300	1717	A-154761	UUACAUCUUUUAUUCUAAGCCUU	4278-4300	1810
AD-77125	A-154762	UUUCAAGAUAUUUGUAAAAGA	3789-3809	1718	A-154763	UCUUUUACAAAUAUCUUGAAAUU	3787-3809	1811
AD-77126	A-154764	AUAAGCAUAUUUUGAAAAUGA	7221-7241	1719	A-154765	UCAUUUUCAAAAUAUGCUUAUUA	7219-7241	1812
AD-77127	A-154766	GUUUUCAAUGCUAGUGUUUAA	5553-5573	1720	A-154767	UUAAACACUAGCAUUGAAAACAA	5551-5573	1813
AD-77128	A-154768	CAAUGAAAUACACAAGUAAAA	813-833	1721	A-154769	UUUUACUUGUGUAUUUCAUUGGG	811-833	1814
AD-77129	A-154770	CAAAAGUCCACUGAUGCAAAA	1764-1784	1722	A-154771	UUUUGCAUCAGUGGGACUUUUGUG	1762-1784	1815
AD-77130	A-154772	AACAUAGAAAGUUUCUUCAAA	1409-1429	1723	A-154773	UUUGAAGAAACUUUCUAUGUUUA	1407-1429	1816

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_000618.3	SEQ ID NO
AD-77131	A-154774	AACCUAAUUAGUAACUUUCCA	1465-1485	1724	A-154775	UGGAAAGUUACUAAUUAGGUUGC	1463-1485	1817
AD-77132	A-154776	GACUUAUUUCCUUCACUUAAA	3442-3462	1725	A-154777	UUUAAGUGAAGGAAAUAAGUCAU	3440-3462	1818
AD-77133	A-154778	GGCUCUCAAACUUAGCAAAAA	4614-4634	1726	A-154779	UUUUUGCUAAGUUUGAGAGCCAA	4612-4634	1819
AD-77134	A-154780	ACAAAGAUAAGUGAAAAGAGA	2312-2332	1727	A-154781	UCUCUUUUCACUUAUCUUUGUAU	2310-2332	1820
AD-77135	A-154782	GUAGUGUACUGUUCACCAAAA	4525-4545	1728	A-154783	UUUUGGUGAACAGUACACUACUU	4523-4545	1821
AD-77136	A-154784	UCACCAACUCAUAGCAAAGUA	6663-6683	1729	A-154785	UACUUUGCUAUGAGUUGGUGAGU	6661-6683	1822
AD-77137	A-154786	AAAAUAACUCAUUAUACCAAA	3577-3597	1730	A-154787	UUUGGUAUAAUGAGUUAUUUUCA	3575-3597	1823
AD-77138	A-154788	AAGAAAGAAUCUUUCCAUUCA	5344-5364	1731	A-154789	UGAAUGGAAAGAUUCUUUCUUCU	5342-5364	1824
AD-77139	A-154790	AUUUCAAGAUAUUUGUAAAAA	3788-3808	1732	A-154791	UUUUUACAAAUAUCUUGAAAUUG	3786-3808	1825
AD-77140	A-154792	AGCUGAAACUCUAGAAUUAAA	5908-5928	1733	A-154793	UUUAAUUCUAGAGUUUCAGCUUG	5906-5928	1826
AD-77141	A-154794	AAAGAUAAAAUCUGAUUUAUGA	5394-5414	1734	A-154795	UCAUAAAUCAGAUUUAUCUUUUG	5392-5414	1827
AD-77142	A-154796	UAUUAAAUUAAAUUCUGAUUUGA	7099-7119	1735	A-154797	UCAAUCAGAAUUAAUUUAAUAAA	7097-7119	1828
AD-77143	A-154798	AAGAAUGAGCUUUCAACUCAA	3825-3845	1736	A-154799	UUGAGUUGAAAGCUCAUUCUUAC	3823-3845	1829
AD-77144	A-154800	GUUUGAUAACAUUUAAAAGAA	780-800	1737	A-154801	UUCUUUUAAAUGUUAUCAAACUU	778-800	1830
AD-77145	A-154802	UGUUUUAAACAUAGAAAGUUA	1402-1422	1738	A-154803	UAACUUUCUAUGUUUAAAACAUA	1400-1422	1831
AD-77146	A-154804	UAAAUAACCCAUUCAUAGCAA	2110-2130	1739	A-154805	UUGCUAUGAAUGGGUUAUUUAUA	2108-2130	1832
AD-77147	A-154806	CACAUAGACUCUUUAAAACUA	5196-5216	1740	A-154807	UAGUUUUAAAGAGUCUAUGUGGG	5194-5216	1833
AD-77148	A-154808	AAGUCACAAAGUUAUCUUCUA	2568-2588	1741	A-154809	UAGAAGAUAACUUUGUGUGACUUUU	2566-2588	1834
AD-77149	A-154810	UAAAGAAAGAAUUUAUGAGAA	5011-5031	1742	A-154811	UUCUCAUAAAUUCUUUCUUUAUC	5009-5031	1835
AD-77150	A-154812	GAAAUGUAUGUUUGACUUGUA	5228-5248	1743	A-154813	UACAAGUCAAACAUACAUUUCUA	5226-5248	1836
AD-77151	A-154814	UUUAUAAGACCUUCCUGUUAA	5611-5631	1744	A-154815	UUAACAGGAAGGUCUUAUAAAAU	5609-5631	1837
AD-77152	A-154816	CUGUGAAUGUGUUUUAUCCAA	2739-2759	1745	A-154817	UUGGAUAAAACACAUUCACAGAG	2737-2759	1838
AD-77153	A-154818	UUAAUUCUGAUUGUAUUUGAA	7106-7126	1746	A-154819	UUCAAAUACAAUCAGAAUUAAUU	7104-7126	1839
AD-77154	A-154820	UGUCAUCUACCUACCUCAAAA	1982-2002	1747	A-154821	UUUUGAGGUAGGUAGAUGACAUA	1980-2002	1840
AD-77155	A-154822	CGUGAAAGCAAAACAAUAGGA	1634-1654	1748	A-154823	UCCUAUUGUUUUGCUUUCACGUA	1632-1654	1841
AD-77156	A-154824	AACAACAAUUCUAUAGAUAGA	4438-4458	1749	A-154825	UCUAUCUAUAGAAUUGUUGUUUU	4436-4458	1842

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 000618.3	SEQ ID NO
AD-77157	A-154826	UAAAGUAUUAUUUGAACUUUA	3920-3940	1750	A-154827	UAAAGUUCAAAUAAUACUUUAAU	3918-3940	1843
AD-77158	A-154828	AGAAAGAAUUUAUGAGAAAUA	5014-5034	1751	A-154829	UAUUUCUCAUAAAUUCUUUCUUU	5012-5034	1844
AD-77159	A-154830	AAAUGCACUGAUGUAAAGUAA	6589-6609	1752	A-154831	UUACUUUACACUCAGUGCAUUUUG	6587-6609	1845
AD-77160	A-154832	AUUAAUUCUGAUUGUAUUUGA	7105-7125	1753	A-154833	UCAAAUACAAUCAGAAUUAAUUU	7103-7125	1846
AD-77161	A-154834	CAAUAAUGUUCUAUAGAAAAA	913-933	1754	A-154835	UUUUUCUAUAGAACAUUAUUGAU	911-933	1847
AD-77162	A-154836	GAUUUUCAAUUUGAUUUUGAA	2269-2289	1755	A-154837	UUCAAAAUCAAAUUGAAAAUCAA	2267-2289	1848
AD-77163	A-154840	UUGAUUUUGAAUUCUGCAUUA	2279-2299	1756	A-154841	UAAUGCAGAAUUCAAAAUCAAAU	2277-2299	1849
AD-77164	A-154842	UUUCAAUUUGAUUUUGAAUUA	2272-2292	1757	A-154843	UAAUUCAAAAUCAAAUUGAAAAU	2270-2292	1850
AD-77165	A-154844	GUGUCUGUGUAUCAUGAAAAA	6798-6818	1758	A-154845	UUUUUCAUGAUACACAGACACAG	6796-6818	1851
AD-77166	A-154846	GGUUUUAUGAAUACAAAGAUA	2300-2320	1759	A-154847	UAUCUUUGUAUUCAUAAAACCAA	2298-2320	1852
AD-77167	A-154848	GCAGAUCAAGAUUUUCUCAUA	2837-2857	1760	A-154849	UAUGAGAAAAUCUUGAUCUGCAG	2835-2857	1853
AD-77168	A-154850	AACUAAUUGAAUCAUUAUCUA	7186-7206	1761	A-154851	UAGAUAAUGAUUCAAUUAGUUAC	7184-7206	1854
AD-77169	A-154852	UGGUUUAUGAAUUGUUUCCUA	1077-1097	1762	A-154853	UAGGAAACAAUUCAUAAACCACU	1075-1097	1855
AD-77170	A-154854	UAGUAUAAUGGUGCUAUUUUA	1574-1594	1763	A-154855	UAAAAUAGCACCAUUAUACUAAA	1572-1594	1856
AD-77171	A-154856	CUGUUUAAUAAGCAUAUUUUA	7214-7234	1764	A-154857	UAAAAUAUGCUUAUUAAACAGUA	7212-7234	1857
AD-77172	A-154858	AAAUAUCAAAACCUUUCAAAA	6828-6848	1765	A-154859	UUUUGAAAGUUUUGAUAUUUUG	6826-6848	1858
AD-77173	A-154860	ACAGAUGUAAAAGAAACUAUA	3207-3227	1766	A-154861	UAUAGUUUCUUUUACAUCUGUCC	3205-3227	1859
AD-77174	A-154862	AACUUUGAGGCCAAUCAUUUA	1373-1393	1767	A-154863	UAAAUGAUUGGCCUCAAAGUUGC	1371-1393	1860

Table 16. IGF-1 in vitro 10nM and 0.1nM screen

Duplex Name	10nM AVG	10nM STD	0.1nM AVG	0.1nM STD	Position in NM_ 000618.3
AD-75740	2.9	1.1	20.9	1.6	7179-7201
AD-75741	9.2	1.5	55.6	9.3	4360-4382
AD-75747	50.3	6.8	67.7	8.8	3763-3785
AD-75748	8.4	1.1	36	10.1	5221-5243
AD-75749	7.8	1.3	65.9	3	6510-6532
AD-75750	4.4	1.3	48	9.3	6829-6851
AD-75751	5.7	1.4	51.7	9.4	822-844
AD-75755	29.4	7.2	60.9	3.8	1408-1430
AD-75757	32.4	5.7	62.3	7.9	3120-3142
AD-75759	9.8	4.3	86.5	11.1	6767-6789
AD-75760	32.2	7.8	56.2	7.1	1083-1105
AD-75761	3.5	1.3	53.3	4.3	7069-7091
AD-75765	7.6	1.9	56.5	8.6	4630-4652
AD-75766	3.3	2.4	35.4	7.4	7188-7210
AD-75769	12.4	3.1	33.6	3.9	906-928
AD-75772	36.1	5.2	79.9	19.3	3211-3233
AD-75774	14	1.2	51.8	7.8	5171-5193
AD-75776	41.1	11.6	84.2	10.3	2735-2757
AD-75778	42.3	7.4	56.4	6.6	1436-1458
AD-75779	8.7	1.6	53.7	9.1	5720-5742
AD-75787	59.5	5.5	96.5	6.2	295-317
AD-75787	57.5	12.9	99.5	10.4	295-317
AD-75788	38.5	5.2	82.2	9.3	434-456
AD-75788	28.1	2.1	88.3	4.4	434-456
AD-75789	58	11.5	81.6	3	302-324
AD-75789	65.1	16.2	93.6	5.6	302-324
AD-75790	54.7	5.9	90.1	1.7	423-445
AD-75790	64.1	6.1	92.6	4.3	423-445
AD-75791	17.4	5	78.8	3.9	395-417
AD-75791	19.5	2.1	87.5	7.1	395-417
AD-75792	12.7	1.5	63.7	6.2	393-415
AD-75792	13.9	2	77.8	12.9	393-415
AD-75793	23.6	4.1	78.5	4.7	391-413
AD-75793	27.8	4.1	90.9	11.6	391-413
AD-75794	40.6	3.1	89	12.3	388-410
AD-75794	46.7	4.4	91.1	9.6	388-410
AD-75795	32.2	8.2	75.2	6.3	389-411

Duplex Name	10nM AVG	10nM STD	0.1nM AVG	0.1nM STD	Position in NM_ 000618.3
AD-75795	27.3	5.3	77.7	7.3	389-411
AD-77120	17.7	1.8	83.5	3.7	6584-6606
AD-77121	28.3	3.4	69	5.9	1007-1029
AD-77122	19.3	6.4	80.4	10.5	6323-6345
AD-77123	21.2	4.1	46.9	8	830-852
AD-77124	13.9	1.5	35.3	5.8	4278-4300
AD-77125	44.5	10.3	52.4	6.9	3787-3809
AD-77126	4.5	3	23.6	3.9	7219-7241
AD-77127	8.3	1.5	43.1	4.9	5551-5573
AD-77128	13.6	4.1	46.6	5.3	811-833
AD-77129	68.5	7.6	99.9	8.2	1762-1784
AD-77130	32.1	5.8	41.4	3.4	1407-1429
AD-77131	36	8.5	53.8	7.2	1463-1485
AD-77132	44.3	8.2	71.7	5.1	3440-3462
AD-77133	21.4	6.4	88.9	5.8	4612-4634
AD-77134	37.9	2.4	82.4	8.7	2310-2332
AD-77135	19.6	5	91.8	17	4523-4545
AD-77136	32.3	8	89.5	3.5	6661-6683
AD-77137	36.8	3.3	67.7	7.7	3575-3597
AD-77138	2.6	1.9	71.3	6.3	5342-5364
AD-77139	45.6	2.5	70.4	8.2	3786-3808
AD-77140	17.1	3.2	65.4	6.1	5906-5928
AD-77141	56.3	17.6	112.7	8.2	5392-5414
AD-77142	18.1	3.7	65.7	8.4	7097-7119
AD-77143	45.7	10.1	93.6	11	3823-3845
AD-77144	5.5	2	43.5	7.6	778-800
AD-77145	59.6	11.1	43.8	5.1	1400-1422
AD-77146	27.7	5.8	64.8	7.7	2108-2130
AD-77147	6.6	5.4	75	10.2	5194-5216
AD-77148	37.6	6.7	65.9	8	2566-2588
AD-77149	5.5	0.7	35.8	5.2	5009-5031
AD-77150	1.1	2.3	48.2	5.1	5226-5248
AD-77151	9.1	2.3	69.2	5.9	5609-5631
AD-77152	52.6	5.9	93.7	6.5	2737-2759
AD-77153	10.4	1	37.8	6.1	7104-7126
AD-77154	57.5	14.5	101.7	11.5	1980-2002
AD-77155	63.3	8.5	53	3.9	1632-1654
AD-77156	14.8	3	47.9	8.9	4436-4458

Duplex Name	10nM AVG	10nM STD	0.1nM AVG	0.1nM STD	Position in NM_ 000618.3
AD-77158	1.3	1	44.3	0.9	5012-5034
AD-77159	20.5	4.9	92.3	17.2	6587-6609
AD-77160	10.8	5.2	39.3	4.9	7103-7125
AD-77161	26.1	1.8	47.3	6.8	911-933
AD-77162	51.6	5.4	93.6	3.1	2267-2289
AD-77163	41.2	4.4	70	3.1	2277-2299
AD-77164	58.5	5.8	95.5	10.9	2270-2292
AD-77165	11.1	2	61.7	7.1	6796-6818
AD-77166	40.3	5.6	74.2	1.7	2298-2320
AD-77167	55	9.2	84.6	18	2835-2857
AD-77168	7.9	0.6	28.4	3.6	7184-7206
AD-77169	36.1	7.5	51.6	5.6	1075-1097
AD-77170	44.4	8.8	66.7	3.6	1572-1594
AD-77171	13.1	1.6	50.1	9.3	7212-7234
AD-77172	38.8	5.4	103.7	12.7	6826-6848
AD-77173	42.4	2.8	79.8	15.2	3205-3227
AD-77174	48.9	3.5	83.3	6.5	1371-1393
Mock	100	6.1	100	5.6	

Table 17. Modified Sense and Antisense Strand Sequences of IGF-1 dsRNAs

Duplex Name	Sense Oligo Name	Sense sequence	SEQ ID NO	Antisense Oligo Name	Antisense sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-75740	A-151647	asasuguaAfcUfAfAfuugaaucauaL96	1861	A-151648	VPusAfsugaUfuCfAfauuaGfuUfacauususu	1954	AAAAUGUAACUAAUUGAAUCAUU	2047
AD-75741	A-151649	usasagaaAfgUfAfCfuugacuaaaaL96	1862	A-151650	VPusUfsuuaGfuCfAfaguaCfuUfucuuasasa	1955	UUUAAGAAAGUACUUGACUAAAA	2048
AD-75747	A-151661	csusuuauAfaUfUfCfuugaaugagaL96	1863	A-151662	VPusCfsucaUfuCfAfagaaUfuAfuaaagscsa	1956	UGCUUUAUAAUUCUUGAAUGAGG	2049
AD-75748	A-151663	asgsauagAfaAfUfGfuauguuugaaL96	1864	A-151664	VPusUfscaaAfcAfUfacauUfuCfuaucusasg	1957	CUAGAUAGAAAUGUAUGUUUGAC	2050
AD-75749	A-151665	ususccacAfaUfCfCfuuagaaucuaL96	1865	A-151666	VPusAfsgauUfcUfAfaggaUfuGfuggaasgsg	1958	CCUUCCACAAUCCUUAGAAUCUG	2051
AD-75750	A-151667	usasucaaAfaCfCfUfuucaaauauaL96	1866	A-151668	VPusAfsuauUfuGfAfaaggUfuUfugauasusu	1959	AAUAUCAAAACCUUUCAAAUAUC	2052
AD-75751	A-151669	ascsaaguAfaAfCfAfauuccaacauaL96	1867	A-151670	VPusAfsuguUfgGfAfauguUfuAfcuugusgsu	1960	ACACAAGUAAACAUUCCAACAUU	2053
AD-75755	A-151677	ascsauagAfaAfGfUfuucuucaacaL96	1868	A-151678	VPusGfsuugAfaGfAfaacuUfuCfuaugususu	1961	AAACAUAGAAAGUUUCUUCAACU	2054
AD-75757	A-151681	csasgucaAfcAfAfGfuauuuuaacaL96	1869	A-151682	VPusGfsuuaAfaAfUfacuuGfuUfgacugsasa	1962	UUCAGUCAACAAGUAUUUUAACU	2055
AD-75759	A-151685	csuscaagCfuGfUfCfuacuuacauaL96	1870	A-151686	VPusAfsuguAfaGfUfagacAfgCfuugagsgsu	1963	ACCUCAAGCUGUCUACUUACAUC	2056
AD-75760	A-151687	gsasauugUfuUfCfCfuuauuugcaaL96	1871	A-151688	VPusUfsgcaAfaUfAfaggaAfaCfaauucsasu	1964	AUGAAUUGUUUCCUUAUUUGCAC	2057
AD-75761	A-151689	asuscuguCfuUfAfGfuugaaaagcaL96	1872	A-151690	VPusGfscuuUfuCfAfacuaAfgAfcagausgsu	1965	ACAUCUGUCUUAGUUGAAAAGCA	2058
AD-75765	A-151697	asasuuagCfaAfUfAfuauuauccaaL96	1873	A-151698	VPusUfsggaUfaAfUfauauUfgCfuaauususu	1966	AAAAUUAGCAAUAUAUUAUCCAA	2059
AD-75766	A-151699	asasuugaAfuCfAfUfuaucuuacaaL96	1874	A-151700	VPusUfsguaAfgAfUfaaugAfuUfcaauusasg	1967	CUAAUUGAAUCAUUAUCUUACAU	2060
AD-75769	A-151705	ususuaucAfaUfAfAfuguucuauaaL96	1875	A-151706	VPusUfsauaGfaAfCfauuaUfuGfauaaasasg	1968	CUUUUAUCAAUAAUGUUCUAUAG	2061
AD-75772	A-151711	gsusaaaaGfaAfAfCfuauacaucaaL96	1876	A-151712	VPusUfsgauGfuAfUfaguuUfcUfuuuacsasu	1969	AUGUAAAAGAAACUAUACAUCAU	2062
AD-75774	A-151715	asasugagGfaAfUfAfauaaguuaaaL96	1877	A-151716	VPusUfsuaaCfuUfAfuuauUfcCfucauuscsu	1970	AGAAUGAGGAAUAAUAAGUUAAA	2063
AD-75776	A-151719	csuscuguGfaAfUfGfuguuuuaucaL96	1878	A-151720	VPusGfsauaAfaAfCfacauUfcAfcagagsasg	1971	CUCUCUGUGAAUGUGUUUUAUCC	2064
AD-75778	A-151723	gsusuccuUfcAfAfAfugaugaguuaL96	1879	A-151724	VPusAfsacuCfaUfCfauuuGfaAfggaacsusc	1972	GAGUUCCUUCAAAUGAUGAGUUA	2065
AD-75779	A-151725	csasggauAfaAfGfAfuaucaauuuaL96	1880	A-151726	VPusAfsaauUfgAfUfaucuUfuAfuccugsusa	1973	UACAGGAUAAAGAUAUCAAUUUA	2066
AD-75787	A-151741	csasugucCfuCfCfUfcgcaucucuaL96	1881	A-151742	VPusAfsgagAfuGfCfgaggAfgGfacaugsgsu	1974	ACCAUGUCCUCCUCGCAUCUCUU	2067
AD-75787	A-151741	csasugucCfuCfCfUfcgcaucucuaL96	1882	A-151742	VPusAfsgagAfuGfCfgaggAfgGfacaugsgsu	1975	ACCAUGUCCUCCUCGCAUCUCUU	2068
AD-75788	A-151743	ususcaacAfaGfCfCfcacaggguaaL96	1883	A-151744	VPusUfsaccCfuGfUfgggcUfuGfuugaasasu	1976	AUUUCAACAAGCCCACAGGGUAU	2069
AD-75788	A-151743	ususcaacAfaGfCfCfcacaggguaaL96	1884	A-151744	VPusUfsaccCfuGfUfgggcUfuGfuugaasasu	1977	AUUUCAACAAGCCCACAGGGUAU	2070
AD-75789	A-151745	uscscucgCfaUfCfUfcuucuaccuaL96	1885	A-151746	VPusAfsgguAfgAfAfgagaUfgCfgaggasgsg	1978	CCUCCUCGCAUCUCUUCUACCUG	2071

Duplex Name	Sense Oligo Name	Sense sequence	SEQ ID NO	Antisense Oligo Name	Antisense sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-75789	A-151745	uscscucgCfaUfCfUfcuucuacccuaL96	1886	A-151746	VPusAfsgguAfgAfAfgagaUfgCfgaggasgsg	1979	CCUCCUCGCAUCUCUUCUACCUG	2072
AD-75790	A-151747	gsgsggcuUfuUfAfUfuucaacaagaL96	1887	A-151748	VPusCfsuugUfuGfAfaauuAfaAfgccccsusg	1980	CAGGGGCUUUUAUUUCAACAAGC	2073
AD-75790	A-151747	gsgsggcuUfuUfAfUfuucaacaagaL96	1888	A-151748	VPusCfsuugUfuGfAfaauuAfaAfgccccsusg	1981	CAGGGGCUUUUAUUUCAACAAGC	2074
AD-75791	A-151749	gsasugcuCfuUfCfAfguucgugugaL96	1889	A-151750	VPusCfsacaCfgAfAfcugaAfgAfgcaucscsa	1982	UGGAUGCUCUUCAGUUCGUGUGU	2075
AD-75791	A-151749	gsasugcuCfuUfCfAfguucgugugaL96	1890	A-151750	VPusCfsacaCfgAfAfcugaAfgAfgcaucscsa	1983	UGGAUGCUCUUCAGUUCGUGUGU	2076
AD-75792	A-151751	usgsgaugCfuCfUfUfcaguucgugaL96	1891	A-151752	VPusCfsacgAfaCfUfgaagAfgCfauccascsc	1984	GGUGGAUGCUCUUCAGUUCGUGU	2077
AD-75792	A-151751	usgsgaugCfuCfUfUfcaguucgugaL96	1892	A-151752	VPusCfsacgAfaCfUfgaagAfgCfauccascsc	1985	GGUGGAUGCUCUUCAGUUCGUGU	2078
AD-75793	A-151753	gsgsuggaUfgCfUfCfuucaguucgaL96	1893	A-151754	VPusCfsgaaCfuGfAfagagCfaUfccaccsasg	1986	CUGGUGGAUGCUCUUCAGUUCGU	2079
AD-75793	A-151753	gsgsuggaUfgCfUfCfuucaguucgaL96	1894	A-151754	VPusCfsgaaCfuGfAfagagCfaUfccaccsasg	1987	CUGGUGGAUGCUCUUCAGUUCGU	2080
AD-75794	A-151755	gscsugguGfgAfUfGfcucuucaguaL96	1895	A-151756	VPusAfscugAfaGfAfgcauCfcAfccagcsusc	1988	GAGCUGGUGGAUGCUCUUCAGUU	2081
AD-75794	A-151755	gscsugguGfgAfUfGfcucuucaguaL96	1896	A-151756	VPusAfscugAfaGfAfgcauCfcAfccagcsusc	1989	GAGCUGGUGGAUGCUCUUCAGUU	2082
AD-75795	A-151757	csusgguGfaUfGfCfucuucaguuaL96	1897	A-151758	VPusAfsacuGfaAfGfagcaUfcCfaccagscsu	1990	AGCUGGUGGAUGCUCUUCAGUUC	2083
AD-75795	A-151757	csusgguGfaUfGfCfucuucaguuaL96	1898	A-151758	VPusAfsacuGfaAfGfagcaUfcCfaccagscsu	1991	AGCUGGUGGAUGCUCUUCAGUUC	2084
AD-77120	A-154752	cscsaaaaUfgCfAfCfuguguaaaaL96	1899	A-154753	VPusUfsuuaCfaUfCfagugCfaUfuuuggsgsc	1992	GCCCAAAAUGCACUGAUGUAAAG	2085
AD-77121	A-154754	uscscaguUfgCfAfCfuaaauuccnaL96	1900	A-154755	VPusAfsggaAfuUfUffaguGfaAfcuggasusc	1993	GAUCCAGUUGCACUAAAUUCCUC	2086
AD-77122	A-154756	csasuucuUfcAfAfCfuaucuuguaaL96	1901	A-154757	VPusUfscaaAfgAfUfaguuGfaAfgaaugsasg	1994	CUCAUUCUUCAACUAUCUUUGAU	2087
AD-77123	A-154758	ascsauucCfaAfCfAfuugucuuuaaL96	1902	A-154759	VPusUfsaaaGfaCfAfaaguUfgGfaaugususu	1995	AAACAUUCCAACAUUGUCUUUAG	2088
AD-77124	A-154760	gsgscuuaGfaAfUfAfaaagauguaaL96	1903	A-154761	VPusUfsacaUfcUfUfuuauUfcUfaagccsusu	1996	AAGGCUUAGAAUAAAAGAUGUAG	2089
AD-77125	A-154762	usususcaaGfaUfAfUfunguaaaaagaL96	1904	A-154763	VPusCfsuuuUfaCfAfaauaUfcUfugaaasusu	1997	AAUUUCAAGAUAUUUGUAAAAGA	2090
AD-77126	A-154764	asusaagcAfuAfUfUfuugaaaaugaL96	1905	A-154765	VPusCfsauuUfuCfAfaauuAfuGfcuuaususa	1998	UAAUAAGCAUAUUUUGAAAAUGU	2091
AD-77127	A-154766	gsusuuucAfaUfGfCfuaguguuuaaL96	1906	A-154767	VPusUfsaaaCfaCfUfagcaUfuGfaaaacsasa	1999	UUGUUUUCAAUGCUAGUGUUUAA	2092
AD-77128	A-154768	csasaugaAfaUfAfCfacaaguaaaaL96	1907	A-154769	VPusUfsuuaCfuUfGfuguaUfuUfcauugsgsg	2000	CCCAAUGAAAUACACAAGUAAAC	2093
AD-77129	A-154770	csasaaagUfcCfAfCfugaugcaaaaL96	1908	A-154771	VPusUfsuugCfaUfCfagugGfaCfuuuugsusg	2001	CACAAAAGUCCACUGAUGCAAAU	2094
AD-77130	A-154772	asascauaCfaAfAfGfuuucuucaaaL96	1909	A-154773	VPusUfsugaAfgAfAfacuuUfcUfaugususasa	2002	UAAACAUAGAAAGUUUCUUCAAC	2095
AD-77131	A-154774	asasccuaAfuUfAfGfguaacuuuccaL96	1910	A-154775	VPusGfsgaaAfgUfUfacuaAfuUfagguusgsc	2003	GCAACCUAAUUAGUAACUUUCCU	2096
AD-77132	A-154776	gsascuuaUfuUfCfCfuucacuuaaaL96	1911	A-154777	VPusUfsuaaGfuGfAfaggaAfaUfaagucsasu	2004	AUGACUUAUUUCCUUCACUUAAU	2097
AD-77133	A-154778	gsgscucuCfaAfAfCfuuagcaaaaaL96	1912	A-154779	VPusUfsuuuGfcUfAfaguuUfgAfgagcsasa	2005	UUGGCUCUCAAACUUAGCAAAAU	2098

Duplex Name	Sense Oligo Name	Sense sequence	SEQ ID NO	Antisense Oligo Name	Antisense sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-77134	A-154780	ascsaaagAfuAfAfGfugaaaagagaL96	1913	A-154781	VPusCfsucuUfuUfCfacuuAfuCfuuugusasu	2006	AUACAAAGAUAAGUGAAAAGAGA	2099
AD-77135	A-154782	gsusagugUfaCfUfGfuucaccaaaaL96	1914	A-154783	VPusUfsuugGfuGfAfacagUfaCfacuacsusu	2007	AAGUAGUGUACUGUUCACCAAAU	2100
AD-77136	A-154784	uscsaccaAfcUfCfAfuagcaaaguaL96	1915	A-154785	VPusAfscuuUfgCfUfaugaGfuUfgggasgsu	2008	ACUCACCAACUCAUAGCAAAGUC	2101
AD-77137	A-154786	asasaauaAfcUfCfAfuuauaccaaaL96	1916	A-154787	VPusUfsuggUfaUfAfaugaGfuUfauuuuscsa	2009	UGAAAAUAACUCAUUAUACCAAU	2102
AD-77138	A-154788	asasgaaaGfaAfUfCfuuuccauucaL96	1917	A-154789	VPusGfsaauGfgAfAfagauUfcUfuucuuscsu	2010	AGAAGAAAGAAUCUUUCCAUUCA	2103
AD-77139	A-154790	asusuucaAfgAfUfAfuuuguaaaaaL96	1918	A-154791	VPusUfsuuuAfcAfAfauauCfuUfgaaaususg	2011	CAAUUUCAAGAUAUUUGUAAAAG	2104
AD-77140	A-154792	asgscugaAfaCfUfCfuagaauuaaaL96	1919	A-154793	VPusUfsuaaUfuCfUfagagUfuUfcagcususg	2012	CAAGCUGAAACUCUAGAAUUAAA	2105
AD-77141	A-154794	asasagauAfaAfUfCfugauuuaugaL96	1920	A-154795	VPusCfsauaAfaUfCfagauUfuAfucuuususg	2013	CAAAAGAUAAAUCUGAUUUAUGC	2106
AD-77142	A-154796	usasuuaaAfuUfAfUfAfuucugauuugaL96	1921	A-154797	VPusCfsaauCfaGfAfauuaAfuUfuaauasasa	2014	UUUAUUAAAUUAAUUCUGAUUGU	2107
AD-77143	A-154798	asasgaauGfaGfCfUfuucaacucaaL96	1922	A-154799	VPusUfsgagUfuGfGfAfaagcUfcAfuucuusasc	2015	GUAAGAAUGAGCUUUCAACUCAU	2108
AD-77144	A-154800	gsusuugaUfaAfCfAfuuuaaaagaaL96	1923	A-154801	VPusUfscuuUfuAfAfauguUfaUfcaaacsusu	2016	AAGUUUGAUAACAUUUAAAAGAU	2109
AD-77145	A-154802	usgsuuuuAfaAfCfAfUfagaaaguuaL96	1924	A-154803	VPusAfsacuUfuCfUfaugUfuAfAfaaacasusa	2017	UAUGUUUUAAACAUAGAAAGUUU	2110
AD-77146	A-154804	usasaauaAfcCfCfAfuucauagcaaL96	1925	A-154805	VPusUfsgcuAfuGfAfauggGfuUfauuuasusa	2018	UAUAAAUAACCCAUUCAUAGCAU	2111
AD-77147	A-154806	csascauaGfaCfUfCfuuuaaaacuaL96	1926	A-154807	VPusAfsguuUfuAfAfagagUfcUfaugugsgsg	2019	CCCACAUAGACUCUUUAAAACUA	2112
AD-77148	A-154808	asasgucaCfaAfAfGfuuaucucuaaL96	1927	A-154809	VPusAfsgaaGfaUfAfacuuUfgUfgacuususu	2020	AAAAGUCACAAAGUUAUCUCUUU	2113
AD-77149	A-154810	usasaagaAfaGfAfAfuuuaggagaaL96	1928	A-154811	VPusUfscucAfuAfAfauucUfuUfcuuuasusc	2021	GAUAAAGAAAGAAUUUAUGAGAA	2114
AD-77150	A-154812	gsasaaugUfaUfGfUfuugacuugaaL96	1929	A-154813	VPusAfscadGfuCfAfaacaUfaCfauuucsusa	2022	UAGAAAUGUAUGUUUGACUUGUU	2115
AD-77151	A-154814	ususuauaAfgAfCfCfuuccuguuaaL96	1930	A-154815	VPusUfsaacAfgGfAfagguCfuUfauaaasasu	2023	AUUUUAUAAGACCUUCCUGUUAG	2116
AD-77152	A-154816	csusgugaAfuGfUfCfguuuauccaaL96	1931	A-154817	VPusUfsggaUfaAfAfacacAfuUfcacagsasg	2024	CUCUGUGAAUGUGUUUAUCCAU	2117
AD-77153	A-154818	ususaauuCfuGfAfUfuguauuugaaL96	1932	A-154819	VPusUfscaaAfuAfUfCfaaucAfgAfauuaasusu	2025	AAUUAAUUCUGAUUGUAUUUGAA	2118
AD-77154	A-154820	usgsucauCfuAfCfCfuaccucaaaaL96	1933	A-154821	VPusUfsugAfgGfUfagguAfgAfugacasusa	2026	UAUGUCAUCUACCUACCUCAAAG	2119
AD-77155	A-154822	csgsugaaAfgCfAfAfaacaauaggaL96	1934	A-154823	VPusCfscuaUfuGfUfuuugCfuUfucacgsusa	2027	UACGUGAAAGCAAAACAAUAGGG	2120
AD-77156	A-154824	asascaacAfaUfUfCfuuauaguagaL96	1935	A-154825	VPusCfsuauCfuAfUfagaaUfuGfuuguususu	2028	AAAACAACAAUUCUAUAGAUAGA	2121
AD-77157	A-154826	usasaaguAfuUfAfUfuugaacuuuaL96	1936	A-154827	VPusAfsaagUfuCfAfaauaAfuAfcuuuasasu	2029	AUUAAAGUAUUAUUUGAACUUUU	2122
AD-77158	A-154828	asgsaaagAfaUfUfUfUfagagaaaauaL96	1937	A-154829	VPusAfsuuuCfuCfAfuaaaUfuCfuuucususu	2030	AAAGAAAGAAUUUAUGAGAAAUU	2123
AD-77159	A-154830	asasaugcAfcUfGfAfuguaaaguaaL96	1938	A-154831	VPusUfsacuUfuAfCfaucaGfuGfcauuususg	2031	CAAAAUGCACUGAUGUAAAGUAG	2124
AD-77160	A-154832	asusuaauUfcUfGfAfuguauuugaL96	1939	A-154833	VPusCfsaaaUfaCfAfaucaGfaAfuuaaususu	2032	AAAUUAAUUCUGAUUGUAUUUGA	2125

Duplex Name	Sense Oligo Name	Sense sequence	SEQ ID NO	Antisense Oligo Name	Antisense sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-77161	A-154834	csasauaaaUfgUfUfCfuauagaaaaaL96	1940	A-154835	VPusUfsuuuCfuAfUfagaaCfaUfuauugsasu	2033	AUCAAUAAAUGUUCUAUAGAAAAG	2126
AD-77162	A-154836	gsasuuuuCfaAfUfUfUfugauuuugaaL96	1941	A-154837	VPusUfscaaAfaUfCfaaauUfgAfaaaucsasa	2034	UUGAUUUUUCAAUUUGAUUUUGAA	2127
AD-77163	A-154840	ususgauuUfuGfAfAfuucugcauuaL96	1942	A-154841	VPusAfsaugCfaGfAfauucAfaAfaucaasasu	2035	AUUUGAUUUUGAAUUCUGCAUUU	2128
AD-77164	A-154842	ususucaaUfuUfGfAfuuuugaauuaL96	1943	A-154843	VPusAfsauuCfaAfAfaucaAfaUfugaaasasu	2036	AUUUUCAAUUUGAUUUUGAAUUC	2129
AD-77165	A-154844	gsusgucuGfuGfUfAfucaugaaaaaL96	1944	A-154845	VPusUfsuuuCfaUfGfauacAfcAfgacacsasg	2037	CUGUGUCUGUGUAUCAUGAAAAU	2130
AD-77166	A-154846	gsgsuuuuAfuGfAfAfuacaaaguaL96	1945	A-154847	VPusAfsucuUfuGfUfauucAfuAfaaaccsasa	2038	UUGGUUUUAUGAAUACAAAGAUA	2131
AD-77167	A-154848	gscsaguCfaAfGfAfuuuucucauaL96	1946	A-154849	VPusAfsugaGfaAfAfaucuUfgAfucugcsasg	2039	CUGCAGAUCAAGAUUUUCUCAUU	2132
AD-77168	A-154850	asascuaaUfuGfAfAfucauuaucuaL96	1947	A-154851	VPusAfsgauAfaUfGfauucAfaUfuagnusasc	2040	GUAACUAAUUGAAUCAUUAUCUU	2133
AD-77169	A-154852	usgsguuuAfuGfAfAfuuguuuccuaL96	1948	A-154853	VPusAfsggaAfaCfAfauucAfuAfaaccascsu	2041	AGUGGUUUAUGAAUUGUUUCCUU	2134
AD-77170	A-154854	usasguauAfaUfGfGfugcuauuuuaL96	1949	A-154855	VPusAfsaaaUfaGfCfaccaUfuAfuacuasasa	2042	UUUAGUAUAAUGGUGCUAUUUUG	2135
AD-77171	A-154856	csusguuuAfaUfAfAfAfgcauauuuuaL96	1950	A-154857	VPusAfsaaaUfaUfUfGfcuuaUfuAfaacagsusa	2043	UACUGUUUAAUAAAGCAUAUUUUG	2136
AD-77172	A-154858	asasauauuCfaAfAfAfAfccuuucaaaaL96	1951	A-154859	VPusUfsuugAfaAfGfguuuUfgAfuauuususg	2044	CAAAAUAUCAAAAACCUUUCAAAU	2137
AD-77173	A-154860	ascsagauGfuAfAfAfAfagaaacuauaL96	1952	A-154861	VPusAfsuagUfuUfCfuuuuAfcAfucuguscsc	2045	GGACAGAUGUAAAAAGAAACUAUA	2138
AD-77174	A-154862	asascuuuGfaGfGfCfcaaucauuuaL96	1953	A-154863	VPusAfsaauGfaUfUfggccUfcAfaaguusgsc	2046	GCAACUUUGAGGCCAAUCAUUUU	2139

Example 11 – Further IGF-1 Transcripts, siRNA Design, and siRNA Screening

Bioinformatics:

A set of siRNAs targeting human IGF1 (human insulin like growth factor 1, NCBI refseqID: NM_000618; NCBI GeneID: 3479) were designed using custom R and Python scripts. The human IGF1 REFSEQ mRNA has a length of 7366 bases.

The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every potential 19mer siRNA from position 10 through the end was determined with a linear model derived the direct measure of mRNA knockdown from more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. The custom Python script built the set of siRNAs by systematically selecting a siRNA every 11 bases along the target mRNA starting at position 10. At each of the positions, the neighboring siRNA (one position to the 5' end of the mRNA, one position to the 3' end of the mRNA) was swapped into the design set if the predicted efficacy was better than the efficacy at the exact every-11th siRNA. Low complexity siRNAs, *i.e.*, those with Shannon Entropy measures below 1.35 were excluded from the set.

In vitro Dual-Glo® screening

Cell culture and transfections

Cos7 cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in DMEM (ATCC) supplemented with 10% FBS, before being released from the plate by trypsinization. Three human IGF-1 Dual-Glo® Luciferase constructs were generated using the psiCHECK2 vector. Construct one contained sequence based on NM_001111285, while constructs two and three contained sequence based on NM_000618 as provided in the prior Example. Dual-luciferase plasmids were co-transfected with siRNA into 5000 cells using Lipofectamine RNAiMax (Invitrogen, Carlsbad CA. cat # 13778-150). For each well of a 384 well plate, 0.1µl of Lipofectamine was added to 15ng of plasmid vector and siRNA in 15µl of Opti-MEM and allowed to complex at room temperature for 15 minutes. The mixture was then added to the cells resuspended in 35ul of fresh complete media. Cells were incubated for 48 hours before luciferase was measured. Single dose experiments were performed at 10nM final duplex concentration.

Dual-Glo® Luciferase assay

Forty-eight hours after the siRNAs were transfected, Firefly (transfection control) and Renilla (fused to IGF1 target sequence in 3' UTR) luciferase were measured. First, media was removed from cells. Then Firefly luciferase activity was measured by adding 20ul of Dual-Glo® Luciferase Reagent mixed with 20ul of complete media to each well. The mixture was incubated at room temperature for 30 minutes before luminescense (500nm) was measured on a Spectramax (Molecular Devices) to detect the Firefly luciferase signal. Renilla luciferase activity was measured by adding 20ul of room temperature of Dual-Glo® Stop & Glo® Reagent to each well and the plates were incubated for 20 minutes before luminescence was again measured to determine the Renilla luciferase signal. The Dual-Glo® Stop & Glo® Reagent quenched the firefl y luciferase signal and sustained luminescence

for the Renilla luciferase reaction. siRNA activity was determined by normalizing the Renilla (IGF1) signal to the Firefly (control) signal within each well. The magnitude of siRNA activity was then assessed relative to cells that were transfected with the same vector but were not treated with siRNA or were treated with a non-targeting siRNA. All transfections were done in quadruplicates.

Table 18. Unmodified Sense and Antisense Strand Sequences of IGF-1 dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 000618.3	SEQ ID NO
AD-74963	A-150432	UAGAUAAAUGUGAGGAUUU	6-24	2140	A-150433	AAAUCCUCACAUUUAUCUA	6-24	2420
AD-74964	A-150434	UUCUCUAAAUCCCUCUUCU	24-42	2141	A-150435	AGAAGAGGGAUUUAGAGAA	24-42	2421
AD-74965	A-150436	CUGUUUGCUAAAUCUCACU	41-59	2142	A-150437	AGUGAGAUUUAGCAAACAG	41-59	2422
AD-74966	A-150438	CUCACUGUCACUGCUAAAU	54-72	2143	A-150439	AUUUAGCAGUGACAGUGAG	54-72	2423
AD-74967	A-150440	UUCAGAGCAGAUAGAGCCU	72-90	2144	A-150441	AGGCUCUAUCUGCUCUGAA	72-90	2424
AD-74968	A-150442	CAUUGCUCUCAACAUCUCA	127-145	2145	A-150443	UGAGAUGUUGAGAGCAAUG	127-145	2425
AD-74969	A-150444	ACCAAUUCAUUUUCAGACU	185-203	2146	A-150445	AGUCUGAAAAUGAAUUGGU	185-203	2426
AD-74970	A-150446	UUUGUACUUCAGAAGCAAU	203-221	2147	A-150447	AUUGCUUCUGAAGUACAAA	203-221	2427
AD-74971	A-150448	AUGGGAAAAAUCAGCAGUA	220-238	2148	A-150449	UACUGCUGAUUUUUCCCAU	220-238	2428
AD-74972	A-150450	CAAUUAUUUAAGUGCUGCU	247-265	2149	A-150451	AGCAGCACUUAAAUAAUUG	247-265	2429
AD-74973	A-150452	UUGAAGGUGAAGAUGCACA	277-295	2150	A-150453	UGUGCAUCUUCACCUUCAA	277-295	2430
AD-74974	A-150454	UUUUAUUUCAACAAGCCCA	430-448	2151	A-150455	UGGGCUUGUUGAAAUAAAA	430-448	2431
AD-74975	A-150456	CACAGGGUAUGGCUCCAGA	447-465	2152	A-150457	UCUGGAGCCAUACCCUGUG	447-465	2432
AD-74976	A-150458	CAGCAGUCGGAGGGCGCCU	462-480	2153	A-150459	AGGCGCCCUCCGACUGCUG	462-480	2433
AD-74977	A-150460	UUGCGCACCCCUCAAGCCU	543-561	2154	A-150461	AGGCUUGAGGGGUGCGCAA	543-561	2434
AD-74978	A-150462	UGCAGGAAACAAGAACUAA	654-672	2155	A-150463	UUAGUUCUUGUUUCCUGCA	654-672	2435
AD-74979	A-150464	CAGGAUGUAGGAAGACCCU	672-690	2156	A-150465	AGGGUCUUCCUACAUCCUG	672-690	2436
AD-74980	A-150466	UUAAACUUUGGAACACCUA	750-768	2157	A-150467	UAGGUGUUCCAAAGUUUAA	750-768	2437
AD-74981	A-150468	AAAUAAGUUUGAUAACAUU	774-792	2158	A-150469	AAUGUUAUCAAACUUAUUU	774-792	2438
AD-74982	A-150470	UUAAAAGAUGGGCGUUUCA	792-810	2159	A-150471	UGAAACGCCCAUCUUUUAA	792-810	2439
AD-74983	A-150472	AAAUACACAAGUAAACAUU	818-836	2160	A-150473	AAUGUUUACUUGUGUAUUU	818-836	2440
AD-74984	A-150474	UUCCAACAUUGUCUUUAGA	835-853	2161	A-150475	UCUAAAGACAAUGUUGGAA	835-853	2441
AD-74985	A-150476	GGAGUGAUUUGCACCUUGA	852-870	2162	A-150477	UCAAGGUGCAAAUCACUCC	852-870	2442
AD-74986	A-150478	AUUGCUGUUGAUCUUUUAU	894-912	2163	A-150479	AUAAAAGAUCAACAGCAAU	894-912	2443
AD-74987	A-150480	UCAAUAAUGUUCUAUAGAA	912-930	2164	A-150481	UUCUAUAGAACAUUAUUGA	912-930	2444

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 000618.3	SEQ ID NO
AD-74988	A-150482	AAAGAAAAAAAAAAUAUAU	930-948	2165	A-150483	AUAUAUUUUUUUUUUCUUU	930-948	2445
AD-74989	A-150484	AUAUAUAUAUAUAUCUUAA	947-965	2166	A-150485	UUAAGAUAUAUAUAUAUAU	947-965	2446
AD-74990	A-150486	UUUCCUUAUUUGCACUUCU	1091-1109	2167	A-150487	AGAAGUGCAAAUAAGGAAA	1091-1109	2447
AD-74991	A-150488	CUUUCUACACAACUCGGGA	1108-1126	2168	A-150489	UCCCGAGUUGUGUAGAAAG	1108-1126	2448
AD-74992	A-150490	GCUGUUUGUUUUACAGUGU	1125-1143	2169	A-150491	ACACUGUAAAACAAACAGC	1125-1143	2449
AD-74993	A-150492	UUACAGUGUCUGAUAAUCU	1135-1153	2170	A-150493	AGAUUAUCAGACACUGUAA	1135-1153	2450
AD-74994	A-150494	CUGAUAAUCUUGUUAGUCU	1144-1162	2171	A-150495	AGACUAACAAGAUUAUCAG	1144-1162	2451
AD-74995	A-150496	UAUACCCCACCACCUCCCUU	1162-1180	2172	A-150497	AAGGGAGGUGGUGGGUAUA	1162-1180	2452
AD-74996	A-150498	UUGCCGAAUUUGGCCUCCU	1195-1213	2173	A-150499	AGGAGGCCAAAUUCGGCAA	1195-1213	2453
AD-74997	A-150500	GCCGAAUUUGGCCUCCUCA	1197-1215	2174	A-150501	UGAGGAGGCCAAAUUCGGC	1197-1215	2454
AD-74998	A-150502	AAAAGCAGCAGCAAGUCGU	1215-1233	2175	A-150503	ACGACUUGCUGCUGCUUUU	1215-1233	2455
AD-74999	A-150504	GUCAAGAAGCACACCAAUU	1232-1250	2176	A-150505	AAUUGGUGUGCUUCUUGAC	1232-1250	2456
AD-75000	A-150506	AGUUGGAUGCAUUUUAUUU	1293-1311	2177	A-150507	AAAUAAAAUGCAUCCAACU	1293-1311	2457
AD-75001	A-150508	UUAGACACAAAGCUUUAUU	1311-1329	2178	A-150509	AAUAAAGCUUUGUGUCUAA	1311-1329	2458
AD-75002	A-150510	CACAUCAUGCUUACAAAAA	1334-1352	2179	A-150511	UUUUUGUAAGCAUGAUGUG	1334-1352	2459
AD-75003	A-150512	AAGAAUAAUGCAAAUAGUU	1352-1370	2180	A-150513	AACUAUUUGCAUUAUUCUU	1352-1370	2460
AD-75004	A-150514	UGCAACUUUGAGGCCAAUA	1370-1388	2181	A-150515	UAUUGGCCUCAAAGUUGCA	1370-1388	2461
AD-75005	A-150516	CAUUUUUAGGCAUAUGUUU	1388-1406	2182	A-150517	AAACAUAUGCCUAAAAAUG	1388-1406	2462
AD-75006	A-150518	UUAAACAUAGAAAGUUUCU	1406-1424	2183	A-150519	AGAAACUUUCUAUGUUUAA	1406-1424	2463
AD-75007	A-150520	CUUCAACUCAAAAGAGUUA	1423-1441	2184	A-150521	UAACUCUUUUGAGUUGAAG	1423-1441	2464
AD-75008	A-150522	UCCUUCAAAUGAUGAGUUA	1440-1458	2185	A-150523	UAACUCAUCAUUUGAAGGA	1440-1458	2465
AD-75009	A-150524	UUAGUAACUUUCCUCUUUU	1472-1490	2186	A-150525	AAAAGAGGAAAGUUACUAA	1472-1490	2466
AD-75010	A-150526	UUUUUCCAUAUAGAGCACU	1494-1512	2187	A-150527	AGUGCUCUAUAUGGAAAAA	1494-1512	2467
AD-75011	A-150528	CUAUGUAAAUUUAGCAUAU	1511-1529	2188	A-150529	AUAUGCUAAAUUUACAUAG	1511-1529	2468
AD-75012	A-150530	AUCAAUUAUACAGGAUAUA	1528-1546	2189	A-150531	UAUAUCCUGUAUAAUUGAU	1528-1546	2469
AD-75013	A-150532	UUUAGUAUAAUGGUGCUAU	1572-1590	2190	A-150533	AUAGCACCAUUAUACUAAA	1572-1590	2470
AD-75014	A-150534	UUGUUAUAUGAAAGAGUCU	1599-1617	2191	A-150535	AGACUCUUUCAUAUAACAA	1599-1617	2471

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 000618.3	SEQ ID NO
AD-75015	A-150536	ACGGUAAUACGUGAAAGCA	1625-1643	2192	A-150537	UGCUUUCACGUAUUACCGU	1625-1643	2472
AD-75016	A-150538	AAAACAAUAGGGGAAGCCU	1643-1661	2193	A-150539	AGGCUUCCCCUAUUGUUUU	1643-1661	2473
AD-75017	A-150540	UACUGAAAACACCAUCCAU	1690-1708	2194	A-150541	AUGGAUGGUGUUUUCAGUA	1690-1708	2474
AD-75018	A-150542	UUGGGAAAGAAGGCAAAGU	1709-1727	2195	A-150543	ACUUUGCCUUCUUUCCCAA	1709-1727	2475
AD-75019	A-150544	UCAGACACAAAAGUCCACU	1757-1775	2196	A-150545	AGUGGACUUUUGUGUCUGA	1757-1775	2476
AD-75020	A-150546	CGAGUCCAGAGAGGAAACU	1793-1811	2197	A-150547	AGUUUCCUCUCUGGACUCG	1793-1811	2477
AD-75021	A-150548	AAACUGUGGAAUGGAAAAA	1807-1825	2198	A-150549	UUUUUCCAUUCCACACAGUUU	1807-1825	2478
AD-75022	A-150550	AGCAGAAGGCUAGGAAUUU	1825-1843	2199	A-150551	AAAUUCCUAGCCUUCUGCU	1825-1843	2479
AD-75023	A-150552	UUAGCAGUCCCUGGUUUCUU	1843-1861	2200	A-150553	AAGAAACCAGGACUGCUAA	1843-1861	2480
AD-75024	A-150554	CAAAAUGGGGGCAAUAUGU	1966-1984	2201	A-150555	ACAUAUUGCCCCCAUUUUG	1966-1984	2481
AD-75025	A-150556	UUUAAAAAAGAUAAAGAUUA	2016-2034	2202	A-150557	UAAUCUUUAUCUUUUUAAA	2016-2034	2482
AD-75026	A-150558	UCAGAUUUUUUUUACCCUA	2033-2051	2203	A-150559	UAGGGUAAAAAAAAAUCUGA	2033-2051	2483
AD-75027	A-150560	UUUUUUACCCUGGGUUGCU	2040-2058	2204	A-150561	AGCAACCCAGGGUAAAAAA	2040-2058	2484
AD-75028	A-150562	CUGUAAGGGUGCAACAUCA	2057-2075	2205	A-150563	UGAUGUUGCACCCUUACAG	2057-2075	2485
AD-75029	A-150564	CUGAGAUGCAAGGAAUUCU	2090-2108	2206	A-150565	AGAAUUCCUUGCAUCUCAG	2090-2108	2486
AD-75030	A-150566	UUGGUGAAUUGAAUGCUCA	2140-2158	2207	A-150567	UGAGCAUUCAAUUCACCAA	2140-2158	2487
AD-75031	A-150568	UUCUUGUCAGUGAAGCUAU	2170-2188	2208	A-150569	AUAGCUUCACUGACAAGAA	2170-2188	2488
AD-75032	A-150570	AAUAACUGGCCAACUAGUU	2192-2210	2209	A-150571	AACUAGUUGGCCAGUUAUU	2192-2210	2489
AD-75033	A-150572	UGUUAAAAGCUAACAGCUA	2210-2228	2210	A-150573	UAGCUGUUAGCUUUUAACA	2210-2228	2490
AD-75034	A-150574	CAAUCUCUUAAAACACUUU	2228-2246	2211	A-150575	AAAGUGUUUUAAGAGAUUG	2228-2246	2491
AD-75035	A-150576	AAAAUAUGUGGGAAGCAUU	2249-2267	2212	A-150577	AAUGCUUCCCACAUAUUUU	2249-2267	2492
AD-75036	A-150578	UUUGAUUUUCAAUUUGAUU	2266-2284	2213	A-150579	AAUCAAAUUGAAAAUCAAA	2266-2284	2493
AD-75037	A-150580	UUGAAUUCUGCAUUUGGUU	2285-2303	2214	A-150581	AACCAAAUGCAGAAUUCAA	2285-2303	2494
AD-75038	A-150582	UUUAUGAAUACAAAGAUAA	2303-2321	2215	A-150583	UUAUCUUUGUAUUCAUAAA	2303-2321	2495
AD-75039	A-150584	GUGAAAAGAGAGAAAGGAA	2322-2340	2216	A-150585	UUCCUUUCUCUCUUUUCAC	2322-2340	2496
AD-75040	A-150586	AAAGAAAAAGGAGAAAAAC	2340-2358	2217	A-150587	GUUUUUCUCCUUUUUCUUU	2340-2358	2497
AD-75041	A-150588	ACAAAGAGAUUUCUACCAA	2357-2375	2218	A-150589	UUGGUAGAAAUCUCUUUGU	2357-2375	2498

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 000618.3	SEQ ID NO
AD-75042	A-150590	UUGUUAGCACUCACUGACU	2398-2416	2219	A-150591	AGUCAGUGAGUGCUAACAA	2398-2416	2499
AD-75043	A-150592	UACAUAUCUAGUAAAAACCU	2432-2450	2220	A-150593	AGGUUUUACUAGAUAUGUA	2432-2450	2500
AD-75044	A-150594	CUCGUUUAAUACUAUAAAU	2449-2467	2221	A-150595	AUUUAUAGUAUUAAAACGAG	2449-2467	2501
AD-75045	A-150596	UUUAAUACUAUAAAUAAUA	2453-2471	2222	A-150597	UAUUAUUUAUAGUAUUAAA	2453-2471	2502
AD-75046	A-150598	UAUUCUAUUCAUUUUGAAA	2470-2488	2223	A-150599	UUUCAAAAUGAAUAGAAUA	2470-2488	2503
AD-75047	A-150600	UUUGAAAAACACAAUGAUU	2482-2500	2224	A-150601	AAUCAUUGUGUUUUUCAAA	2482-2500	2504
AD-75048	A-150602	AAGGAAAGUGAUCCAAAAU	2521-2539	2225	A-150603	AUUUUGGAUCACUUUCCUU	2521-2539	2505
AD-75049	A-150604	UUUGAAAUAUUAAAAUAAU	2539-2557	2226	A-150605	AUUAUUUUAAUAUUUCAAA	2539-2557	2506
AD-75050	A-150606	UUAAAAUAAUAUCUAAUAA	2548-2566	2227	A-150607	UUAUUAGAUAUUAUUUUAA	2548-2566	2507
AD-75051	A-150608	AAAAGUCACAAAGUUAUCU	2566-2584	2228	A-150609	AGAUAACUUUGUGUGACUUUU	2566-2584	2508
AD-75052	A-150610	UUCUUUAACAAACUUUACU	2584-2602	2229	A-150611	AGUAAAGUUUGUUAAAGAA	2584-2602	2509
AD-75053	A-150612	CUCUUAUUCUUAGCUGUAU	2601-2619	2230	A-150613	AUACAGCUAAGAAUAAGAG	2601-2619	2510
AD-75054	A-150614	AUAUACAUUUUUUUUAAAAG	2618-2636	2231	A-150615	CUUUUAAAAAAAAUGUAUAU	2618-2636	2511
AD-75055	A-150616	CAUUUUUUUAAAAGUUUGU	2623-2641	2232	A-150617	ACAAACUUUUAAAAAAAAUG	2623-2641	2512
AD-75056	A-150618	GUUAAAAAUAUGCUUGACUA	2640-2658	2233	A-150619	UAGUCAAGCAUAUUUUAAC	2640-2658	2513
AD-75057	A-150620	AUGCUUGACUAGAGUUUCA	2648-2666	2234	A-150621	UGAAACUCUAGUCAAGCAU	2648-2666	2514
AD-75058	A-150622	CAGUUGAAAGGCAAAAACU	2666-2684	2235	A-150623	AGUUUUUGCCUUUCAACUG	2666-2684	2515
AD-75059	A-150624	UUCCAUCACAACAAGAAAU	2684-2702	2236	A-150625	AUUUCUUGUUGUGAUGGAA	2684-2702	2516
AD-75060	A-150626	UUGGUAUCAAGAAAGUCCA	2771-2789	2237	A-150627	UGGACUUUCUUGAUACCAA	2771-2789	2517
AD-75061	A-150628	GUUAGUGUACUAGUCCAUA	2793-2811	2238	A-150629	UAUGGACUAGUACACUAAC	2793-2811	2518
AD-75062	A-150630	CAUAGCCUAGAAAAUGAUA	2811-2829	2239	A-150631	UAUCAUUUUCUAGGCUAUG	2811-2829	2519
AD-75063	A-150632	UCCCUAUCUGCAGAUCAAA	2828-2846	2240	A-150633	UUUGAUCUGCAGAUAGGGA	2828-2846	2520
AD-75064	A-150634	UUAUCCAGCAUUCAGAUCU	2869-2887	2241	A-150635	AGAUCUGAAUGCUGGAUAA	2869-2887	2521
AD-75065	A-150636	UUUUUGGUUAAAAGUACCA	2906-2924	2242	A-150637	UGGUACUUUUAACCAAAAA	2906-2924	2522
AD-75066	A-150638	UACCCAGGCUUGAUUAUUU	2920-2938	2243	A-150639	AAAUAAUCAAGCCUGGGUA	2920-2938	2523
AD-75067	A-150640	UCAUGCAAAUUCUAUAUUU	2938-2956	2244	A-150641	AAAUAUAGAAUUUGCAUGA	2938-2956	2524
AD-75068	A-150642	UUACAUUCUUGGAAAGUCU	2956-2974	2245	A-150643	AGACUUUCCAAGAAUGUAA	2956-2974	2525

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 000618.3	SEQ ID NO
AD-75069	A-150644	UCUUGGAAAGUCUAUAUGA	2962-2980	2246	A-150645	UCAUAUAGACUUUCCAAGA	2962-2980	2526
AD-75070	A-150646	AAAAACAAAAAUAAACAUCU	2980-2998	2247	A-150647	AGAUGUUAUUUUUGUUUUU	2980-2998	2527
AD-75071	A-150648	UUCUCCCACUGGUCACCU	3006-3024	2248	A-150649	AGGUGACCCAGUGGGAGAA	3006-3024	2528
AD-75072	A-150650	CAAGGAUCAGAGGCCAGGA	3025-3043	2249	A-150651	UCCUGGCCUCUGAUCCUUG	3025-3043	2529
AD-75073	A-150652	AAAAAAAAAAAAAAGACUA	3043-3061	2250	A-150653	UAGUCUUUUUUUUUUUUUU	3043-3061	2530
AD-75074	A-150654	UCCCUGGAUCUCUGAAUAU	3060-3078	2251	A-150655	AUAUUCAGAGAUCCAGGGA	3060-3078	2531
AD-75075	A-150656	AUAUGCAAAAAGAAGGCCA	3077-3095	2252	A-150657	UGGCCUUCUUUUUGCAUAU	3077-3095	2532
AD-75076	A-150658	UAGUGGAGCCAGCAAUCCU	3100-3118	2253	A-150659	AGGAUUGCUGGCUCCACUA	3100-3118	2533
AD-75077	A-150660	UUAACUCUCAGUCCAACAU	3137-3155	2254	A-150661	AUGUUGGACUGAGAGUUAA	3137-3155	2534
AD-75078	A-150662	UUAUUUGAAUUGAGCACCU	3155-3173	2255	A-150663	AGGUGCUCAAUUCAAAUAA	3155-3173	2535
AD-75079	A-150664	CAGAUGUAAAAGAAACUAU	3208-3226	2256	A-150665	AUAGUUUCUUUUACAUCUG	3208-3226	2536
AD-75080	A-150666	AUACAUCAUUUUUGCCCUA	3225-3243	2257	A-150667	UAGGGCAAAAAUGAUGUAU	3225-3243	2537
AD-75081	A-150668	UUUUGCCCUCUGCCUGUUU	3234-3252	2258	A-150669	AAACAGGCAGAGGGCAAAA	3234-3252	2538
AD-75082	A-150670	UUCCAGACAUACAGGUUCU	3252-3270	2259	A-150671	AGAACCUGUAUGUCUGGAA	3252-3270	2539
AD-75083	A-150672	CUGUGGAAUAAGAUACUGA	3269-3287	2260	A-150673	UCAGUAUCUUAUUCCACAG	3269-3287	2540
AD-75084	A-150674	UAAGAUACUGGACUCCUCU	3277-3295	2261	A-150675	AGAGGAGUCCAGUAUCUUA	3277-3295	2541
AD-75085	A-150676	CUUCCCAAGAUGGCACUUA	3294-3312	2262	A-150677	UAAGUGCCAUCUUGGGAAG	3294-3312	2542
AD-75086	A-150678	GUGUACCUUUUAAAAAUAU	3334-3352	2263	A-150679	AUAAUUUUAAAAGGUACAC	3334-3352	2543
AD-75087	A-150680	UUCCCUCUCAACAAAACUU	3352-3370	2264	A-150681	AAGUUUUGUUGAGAGGGAA	3352-3370	2544
AD-75088	A-150682	UUUAUAGGCAGUCUUCUGA	3369-3387	2265	A-150683	UCAGAAGACUGCCUAUAAA	3369-3387	2545
AD-75089	A-150684	UUUUCUGUCAUAGUUAGAU	3400-3418	2266	A-150685	AUCUAACUAUGACAGAAAA	3400-3418	2546
AD-75090	A-150686	AUGUGAUAAUUCUAAGAGU	3417-3435	2267	A-150687	ACUCUUAGAAUUAUCACAU	3417-3435	2547
AD-75091	A-150688	UUCCUUCACUUAAUUCUAU	3449-3467	2268	A-150689	AUAGAAUUAAGUGAAGGAA	3449-3467	2548
AD-75092	A-150690	AUUAUCUUUCUUAACUUUU	3517-3535	2269	A-150691	AAAAGUUAAGAAAGAUAAU	3517-3535	2549
AD-75093	A-150692	UUCCAACACAUAAUCCUCU	3535-3553	2270	A-150693	AGAGGAUUAUGUGUUGGAA	3535-3553	2550
AD-75094	A-150694	AAAUAAAUUGAAAAUAACU	3567-3585	2271	A-150695	AGUUAUUUUCAAUUUAUUU	3567-3585	2551
AD-75095	A-150696	UCAUUAUACCAAUUCACUA	3585-3603	2272	A-150697	UAGUGAAUUGGUAUAAUGA	3585-3603	2552

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AD-75096	A-150698	AUUUUAUUUUUUAAUGAAU	3603-3621	2273	A-150699	AUUCAUUAAAAAAUAAAAU	3603-3621	2553
AD-75097	A-150700	UUAAAACUAGAAAACAAAU	3621-3639	2274	A-150701	AUUUGUUUUCUAGUUUUAA	3621-3639	2554
AD-75098	A-150702	UUGAUUACUAUAUACUACA	3662-3680	2275	A-150703	UGUAGUAUAUAGUAAUCAA	3662-3680	2555
AD-75099	A-150704	AUGACUCAGAUUUCAUAGA	3686-3704	2276	A-150705	UCUAUGAAAUCUGAGUCAU	3686-3704	2556
AD-75100	A-150706	AAAGGAGCAACCAAAAUGU	3704-3722	2277	A-150707	ACAUUUUGGUUGCUCCUUU	3704-3722	2557
AD-75101	A-150708	GUCACAACCCAAAACUUUA	3721-3739	2278	A-150709	UAAAGUUUUGGGUUGUGAC	3721-3739	2558
AD-75102	A-150710	AAACUUUACAAGCUUUGCU	3732-3750	2279	A-150711	AGCAAAGCUUGUAAAGUUU	3732-3750	2559
AD-75103	A-150712	UUCAGAAUUAGAUUGCUUU	3750-3768	2280	A-150713	AAAGCAAUCUAAUUCUGAA	3750-3768	2560
AD-75104	A-150714	UUAUAAUUCUUGAAUGAGA	3767-3785	2281	A-150715	UCUCAUUCAAGAAUUAUAA	3767-3785	2561
AD-75105	A-150716	UAAUUCUUGAAUGAGGCAA	3770-3788	2282	A-150717	UUGCCUCAUUCAAGAAUUA	3770-3788	2562
AD-75106	A-150718	AUUUCAAGAUAUUUGUAAA	3788-3806	2283	A-150719	UUUACAAAUAUCUUGAAAU	3788-3806	2563
AD-75107	A-150720	AAGAACAGUAAACAUUGGU	3806-3824	2284	A-150721	ACCAAUGUUUACUGUUCUU	3806-3824	2564
AD-75108	A-150722	UUUCAACUCAUAGGCUUAU	3835-3853	2285	A-150723	AUAAGCCUAUGAGUUGAAA	3835-3853	2565
AD-75109	A-150724	UUGACCAUACUGGAUACUU	3865-3883	2286	A-150725	AAGUAUCCAGUAUGGUCAA	3865-3883	2566
AD-75110	A-150726	UUUAAGAUGAGGCAGUUCA	3939-3957	2287	A-150727	UGAACUGCCUCAUCUUAAA	3939-3957	2567
AD-75111	A-150728	CAUCAGAAUCCACUCUUCU	3982-4000	2288	A-150729	AGAAGAGUGGAUUCUGAUG	3982-4000	2568
AD-75112	A-150730	UAGGGAUAUGAAAAUCUCU	4000-4018	2289	A-150731	AGAGAUUUUCAUAUCCCUA	4000-4018	2569
AD-75113	A-150732	UUCACCCUAAGGAUCCAAU	4081-4099	2290	A-150733	AUUGGAUCCUUAGGGUGAA	4081-4099	2570
AD-75114	A-150734	AUGGAAUACUGAAAAGAAA	4098-4116	2291	A-150735	UUUCUUUUCAGUAUUCCAU	4098-4116	2571
AD-75115	A-150736	GAAUACUGAAAAGAAAUCA	4101-4119	2292	A-150737	UGAUUUCUUUUCAGUAUUC	4101-4119	2572
AD-75116	A-150738	ACUUCCUUGAAAAUUUUAU	4119-4137	2293	A-150739	AUAAAAUUUUCAAGGAAGU	4119-4137	2573
AD-75117	A-150740	UUAAAAAACAAACAAACAA	4137-4155	2294	A-150741	UUGUUUGUUUGUUUUUUAA	4137-4155	2574
AD-75118	A-150742	AAACAAAAAGCCUGUCCAA	4154-4172	2295	A-150743	UUGGACAGGCUUUUUGUUU	4154-4172	2575
AD-75119	A-150744	UUUGUGUAGAUGAAACCAU	4208-4226	2296	A-150745	AUGGUUUCAUCUACACAAA	4208-4226	2576
AD-75120	A-150746	UUGGGAGAAGGCUUAGAAU	4271-4289	2297	A-150747	AUUCUAAGCCUUCUCCCAA	4271-4289	2577
AD-75121	A-150748	UAAAAGAUGUAGCACAUUU	4289-4307	2298	A-150749	AAAUGUGCUACAUCUUUUA	4289-4307	2578
AD-75122	A-150750	UUAUUGUUUGGCCAGCUAU	4319-4337	2299	A-150751	AUAGCUGGCCAAACAAUAA	4319-4337	2579

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 000618.3	SEQ ID NO
AD-75123	A-150752	AUGCCAAUGUGGUGCUAUU	4336-4354	2300	A-150753	AAUAGCACCACAUUGGCAU	4336-4354	2580
AD-75124	A-150754	AAUGUGGUGCUAUUGUUUA	4341-4359	2301	A-150755	UAAACAAUAGCACCACAUU	4341-4359	2581
AD-75125	A-150756	CUUUAAGAAAGUACUUGAA	4359-4377	2302	A-150757	UUCAAGUACUUUCUUAAAG	4359-4377	2582
AD-75126	A-150758	CUAAAAAAAAAAGAAAAAA	4377-4395	2303	A-150759	UUUUUUCUUUUUUUUUUAG	4377-4395	2583
AD-75127	A-150760	AAGAAAAAAAAAGAAAGCAU	4395-4413	2304	A-150761	AUGCUUUCUUUUUUUUCUU	4395-4413	2584
AD-75128	A-150762	AUAGACAUAUUUUUUUAAA	4412-4430	2305	A-150763	UUUAAAAAAAAUAUGUCUAU	4412-4430	2585
AD-75129	A-150764	UUAAAGUAUAAAAAACAACA	4426-4444	2306	A-150765	UGUUGUUUUUAUACUUUAA	4426-4444	2586
AD-75130	A-150766	CAAUUCUAUAGAUAGAUGA	4443-4461	2307	A-150767	UCAUCUAUCUAUAGAAUUG	4443-4461	2587
AD-75131	A-150768	GGCUUAAUAAAAUAGCAUU	4460-4478	2308	A-150769	AAUGCUAUUUUAUUAAGCC	4460-4478	2588
AD-75132	A-150770	UAAUAAAAUAGCAUUAGGU	4464-4482	2309	A-150771	ACCUAAUGCUAUUUUAUUA	4464-4482	2589
AD-75133	A-150772	UAUCUAGCCACCACCACCU	4484-4502	2310	A-150773	AGGUGGUGGUGGCUAGAUA	4484-4502	2590
AD-75134	A-150774	UUUAUCACUCACAAGUAGU	4511-4529	2311	A-150775	ACUACUUGUGAGUGAUAAA	4511-4529	2591
AD-75135	A-150776	GGCAGGAGUUGGAAAUUUU	4566-4584	2312	A-150777	AAAAUUUCCAACUCCUGCC	4566-4584	2592
AD-75136	A-150778	UUUAAAGUUAGAAGGCUCA	4584-4602	2313	A-150779	UGAGCCUUCUAACUUUAAA	4584-4602	2593
AD-75137	A-150780	CCAUUGUUUUGUUGGCUCU	4601-4619	2314	A-150781	AGAGCCAACAAAACAAUGG	4601-4619	2594
AD-75138	A-150782	UUAGCAAAAUUAGCAAUAU	4625-4643	2315	A-150783	AUAUUGCUAAUUUUGCUAA	4625-4643	2595
AD-75139	A-150784	AUAUUAUCCAAUCUUCUGA	4642-4660	2316	A-150785	UCAGAAGAUUGGAUAAUAU	4642-4660	2596
AD-75140	A-150786	UUAUCCAAUCUUCUGAACU	4645-4663	2317	A-150787	AGUUCAGAAGAUUGGAUAA	4645-4663	2597
AD-75141	A-150788	AAGAGCAUGGAGAAUAAAC	4669-4687	2318	A-150789	GUUUAUUCUCCAUGCUCUU	4669-4687	2598
AD-75142	A-150790	ACGCGGGAAAAAAGAUCUU	4686-4704	2319	A-150791	AAGAUCUUUUUUCCCGCGU	4686-4704	2599
AD-75143	A-150792	GAUCUUAUAGGCAAAUAGA	4699-4717	2320	A-150793	UCUAUUUGCCUAUAAGAUC	4699-4717	2600
AD-75144	A-150794	AAGAAUUUAAAAGAUAAGU	4717-4735	2321	A-150795	ACUUAUCUUUUAAAUUCUU	4717-4735	2601
AD-75145	A-150796	GUAAGUUCCUUAUUGAUUU	4734-4752	2322	A-150797	AAAUCAAUAAGGAACUUAC	4734-4752	2602
AD-75146	A-150798	UUUUGUGCACUCUGCUCUA	4751-4769	2323	A-150799	UAGAGCAGAGUGCACAAAA	4751-4769	2603
AD-75147	A-150800	AAACAGAUAUUCAGCAAGU	4770-4788	2324	A-150801	ACUUGCUGAAUAUCUGUUU	4770-4788	2604
AD-75148	A-150802	UCAGCAAGUGGAGAAAAUA	4780-4798	2325	A-150803	UAUUUUCUCCACUUGCUGA	4780-4798	2605
AD-75149	A-150804	AAGAACAAAGAGAAAAAAU	4798-4816	2326	A-150805	AUUUUUUCUCUUUGUUCUU	4798-4816	2606

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 000618.3	SEQ ID NO
AD-75150	A-150806	AUACAUAGAUUUACCUGCA	4815-4833	2327	A-150807	UGCAGGUAAAUCUAUGUAU	4815-4833	2607
AD-75151	A-150808	UUACCUGCAAAAAAUAGCU	4825-4843	2328	A-150809	AGCUAUUUUUUGCAGGUAA	4825-4843	2608
AD-75152	A-150810	UUUAUAGAAGACAUUCUCA	4884-4902	2329	A-150811	UGAGAAUGUCUUCUAUAAA	4884-4902	2609
AD-75153	A-150812	AGACAUCUCAAAGAGCAGU	4911-4929	2330	A-150813	ACUGCUCUUUGAGAUGUCU	4911-4929	2610
AD-75154	A-150814	UAUGAGAUGGGGGUUAUCU	4987-5005	2331	A-150815	AGAUAACCCCCAUCUCAUA	4987-5005	2611
AD-75155	A-150816	CUACUGAUAAAGAAAGAAU	5004-5022	2332	A-150817	AUUCUUUCUUUAUCAGUAG	5004-5022	2612
AD-75156	A-150818	AAAGAAUUUAUGAGAAAUU	5016-5034	2333	A-150819	AAUUUCUCAUAAAUUCUUU	5016-5034	2613
AD-75157	A-150820	UAACAAUCUGUGAAGAUUU	5050-5068	2334	A-150821	AAAUCUUCACAGAUUGUUA	5050-5068	2614
AD-75158	A-150822	UUUUACUUUAUACAGUCUU	5098-5116	2335	A-150823	AAGACUGUAUAAAGUAAAA	5098-5116	2615
AD-75159	A-150824	UUUAUGAAUUUCUUAAUGU	5115-5133	2336	A-150825	ACAUUAAGAAAUUCAUAAA	5115-5133	2616
AD-75160	A-150826	UUAAUGUUCAAAAUGACUU	5127-5145	2337	A-150827	AAGUCAUUUUGAACAUUAA	5127-5145	2617
AD-75161	A-150828	UUCUUCUUUUUUAUAUCA	5153-5171	2338	A-150829	UGAUAUAAAAAAAGAAGAA	5153-5171	2618
AD-75162	A-150830	AGAAUGAGGAAUAAUAAGU	5171-5189	2339	A-150831	ACUUAUUAUUCCUCAUUCU	5171-5189	2619
AD-75163	A-150832	UUAAACCCACAUAGACUCU	5189-5207	2340	A-150833	AGAGUCUAUGUGGGUUUAA	5189-5207	2620
AD-75164	A-150834	CUUUAAAACUAUAGGCUAA	5206-5224	2341	A-150835	UUAGCCUAUAGUUUUAAAG	5206-5224	2621
AD-75165	A-150836	AGAUAGAAAUGUAUGUUUA	5223-5241	2342	A-150837	UAAACAUACAUUUCUAUCU	5223-5241	2622
AD-75166	A-150838	UUUGACUUGUUUGAAGCUAU	5238-5256	2343	A-150839	AUAGCUUCAACAAGUCAAA	5238-5256	2623
AD-75167	A-150840	UUUUUAAUCUUAAAAGAUU	5284-5302	2344	A-150841	AAUCUUUUAAGAUUAAAAA	5284-5302	2624
AD-75168	A-150842	UUGUGCUAAUUUAUUAGAA	5301-5319	2345	A-150843	UUCUAAUAAAUUAGCACAA	5301-5319	2625
AD-75169	A-150844	UUAUUAGAGCAGAACCUGU	5311-5329	2346	A-150845	ACAGGUUCUGCUCUAAUAA	5311-5329	2626
AD-75170	A-150846	GUUUGGCUCUCCUCAGAAA	5328-5346	2347	A-150847	UUUCUGAGGAGAGCCAAAC	5328-5346	2627
AD-75171	A-150848	CAAUAUUUUCAAAAGAUAA	5383-5401	2348	A-150849	UUAUCUUUUGAAAAUAUUG	5383-5401	2628
AD-75172	A-150850	UCAAAAGAUAAAUCUGAUU	5391-5409	2349	A-150851	AAUCAGAUUUAUCUUUUGA	5391-5409	2629
AD-75173	A-150852	UUAUGCAAUGGCAUCAUUU	5409-5427	2350	A-150853	AAAUGAUGCCAUUGCAUAA	5409-5427	2630
AD-75174	A-150854	UGCAAUGGCAUCAUUUAUU	5412-5430	2351	A-150855	AAUAAAUGAUGCCAUUGCA	5412-5430	2631
AD-75175	A-150856	UUUAAAACAGAAGAAUUGU	5430-5448	2352	A-150857	ACAAUUCUUCUGUUUUAAA	5430-5448	2632
AD-75176	A-150858	AACAACAAAAGGAAAAUGU	5505-5523	2353	A-150859	ACAUUUUCCUUUUGUUGUU	5505-5523	2633

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 000618.3	SEQ ID NO
AD-75177	A-150860	UUAAUCCUGUAGUACAUAU	5570-5588	2354	A-150861	AUAUGUACUACAGGAUUAA	5570-5588	2634
AD-75178	A-150862	UUUAAUAUUUUAUAAGACA	5603-5621	2355	A-150863	UGUCUUAUAAAAUAUUAAA	5603-5621	2635
AD-75179	A-150864	CCUUCCUGUUAGGUAUUAA	5620-5638	2356	A-150865	UUAAUACCUAACAGGAAGG	5620-5638	2636
AD-75180	A-150866	UUAGGUAUUAGAAAGUGAU	5628-5646	2357	A-150867	AUCACUUUCUAAUACCUAA	5628-5646	2637
AD-75181	A-150868	AUACAUAGAUAUCUUUUUU	5645-5663	2358	A-150869	AAAAAAGAUAUCUAUGUAU	5645-5663	2638
AD-75182	A-150870	UUUUUGUGUAAUUUCUAUU	5659-5677	2359	A-150871	AAUAGAAAUUACACAAAAA	5659-5677	2639
AD-75183	A-150872	UUAAAAAAGAGAGAAGACU	5677-5695	2360	A-150873	AGUCUUCUCUCUUUUUUAA	5677-5695	2640
AD-75184	A-150874	CUGUCAGAAGCUUUAAGUA	5694-5712	2361	A-150875	UACUUAAAGCUUCUGACAG	5694-5712	2641
AD-75185	A-150876	UAUGGUACAGGAUAAAGAU	5715-5733	2362	A-150877	AUCUUUAUCCUGUACCAUA	5715-5733	2642
AD-75186	A-150878	UUAAAUAACCAAUUCCUAU	5740-5758	2363	A-150879	AUAGGAAUUGGUUAUUUAA	5740-5758	2643
AD-75187	A-150880	UUGUUUUUUAAAGAAACCU	5773-5791	2364	A-150881	AGGUUUCUUUAAAAAACAA	5773-5791	2644
AD-75188	A-150882	CUCUCACAGAUAAGACAGA	5790-5808	2365	A-150883	UCUGUCUUAUCUGUGAGAG	5790-5808	2645
AD-75189	A-150884	CAGAAUUUUAUAGAGGGCU	5884-5902	2366	A-150885	AGCCCUCUAUAAAAUUCUG	5884-5902	2646
AD-75190	A-150886	UCUAGAAUUAAAGGAACCU	5917-5935	2367	A-150887	AGGUUCCUUUAAUUCUAGA	5917-5935	2647
AD-75191	A-150888	CUCACUGAAAACAUAUAUU	5934-5952	2368	A-150889	AAUAUAUGUUUUCAGUGAG	5934-5952	2648
AD-75192	A-150890	AAACAUAUAUUUUCACGUGU	5942-5960	2369	A-150891	ACACGUGAAAUAUAUGUUU	5942-5960	2649
AD-75193	A-150892	GUUCCCUCUUUUUUUUUUU	5959-5977	2370	A-150893	AAAAAAAAAAAAGAGGGAAC	5959-5977	2650
AD-75194	A-150894	UUAAGCGAUUCUCCUGCCU	6066-6084	2371	A-150895	AGGCAGGAGAAUCGCUUAA	6066-6084	2651
AD-75195	A-150896	CGGCUAAUUUUUUGGAUUU	6129-6147	2372	A-150897	AAAUCCAAAAAAUUAGCCG	6129-6147	2652
AD-75196	A-150898	UUUAAUAGAGACGGGGUUU	6147-6165	2373	A-150899	AAACCCCGUCUCUAUUAAA	6147-6165	2653
AD-75197	A-150900	UUUACCAUGUUGGCCAGGU	6164-6182	2374	A-150901	ACCUGGCCAACAUGGUAAA	6164-6182	2654
AD-75198	A-150902	UUGCUGGGAUUACAGGCAU	6231-6249	2375	A-150903	AUGCCUGUAAUCCCAGCAA	6231-6249	2655
AD-75199	A-150904	UUAAACAUGAUCCUUCUCU	6303-6321	2376	A-150905	AGAGAAGGAUCAUGUUUAA	6303-6321	2656
AD-75200	A-150906	GGGGUCUUUCAAGGGGAAA	6346-6364	2377	A-150907	UUUCCCCUUGAAAGACCCC	6346-6364	2657
AD-75201	A-150908	AAAAAUCCAAGCUUUUUUA	6364-6382	2378	A-150909	UAAAAAAGCUUGGAUUUUU	6364-6382	2658
AD-75202	A-150910	AAAGUAAAAAAAAAAAAAG	6382-6400	2379	A-150911	CUUUUUUUUUUUUACUUU	6382-6400	2659
AD-75203	A-150912	AGAGAGGACACAAAACCAA	6399-6417	2380	A-150913	UUGGUUUUGUGUCCUCUCU	6399-6417	2660

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 000618.3	SEQ ID NO
AD-75204	A-150914	UUAAGAUGGAGACAGAGUU	6444-6462	2381	A-150915	AACUCUGUCUCCAUCUUAA	6444-6462	2661
AD-75205	A-150916	UUUCUCCUAAUAACCGGAA	6461-6479	2382	A-150917	UUCCGGUUAUUAGGAGAAA	6461-6479	2662
AD-75206	A-150918	GCUGAAUUACCUUUCACUU	6479-6497	2383	A-150919	AAGUGAAAGGUAAUUCAGC	6479-6497	2663
AD-75207	A-150920	UUCAAAAACAUGACCUUCA	6497-6515	2384	A-150921	UGAAGGUCAUGUUUUUGAA	6497-6515	2664
AD-75208	A-150922	CAAUCCUUAGAAUCUGCCU	6517-6535	2385	A-150923	AGGCAGAUUCUAAGGAUUG	6517-6535	2665
AD-75209	A-150924	UUUUAUAUUACUGAGGCCU	6538-6556	2386	A-150925	AGGCCUCAGUAAUAUAAAA	6538-6556	2666
AD-75210	A-150926	AAAAGUAAACAUUACUCAU	6557-6575	2387	A-150927	AUGAGUAAUGUUUACUUUU	6557-6575	2667
AD-75211	A-150928	UUUAUUUUGCCCAAAAUGA	6576-6594	2388	A-150929	UCAUUUUGGGCAAAAUAAA	6576-6594	2668
AD-75212	A-150930	CACUGAUGUAAAGUAGGAA	6594-6612	2389	A-150931	UUCCUACUUUACAUCAGUG	6594-6612	2669
AD-75213	A-150932	AAAAUAAAAACAGAGCUCU	6612-6630	2390	A-150933	AGAGCUCUGUUUUUAUUUU	6612-6630	2670
AD-75214	A-150934	CUAAAAUCCCCUUUCAAGCA	6629-6647	2391	A-150935	UGCUUGAAAGGGAUUUUAG	6629-6647	2671
AD-75215	A-150936	UUGACCCCACUCACCAACU	6653-6671	2392	A-150937	AGUUGGUGAGUGGGGUCAA	6653-6671	2672
AD-75216	A-150938	UUAUCUUGUACCCGCUGCU	6722-6740	2393	A-150939	AGCAGCGGGUACAAGAUAA	6722-6740	2673
AD-75217	A-150940	CUGAAACCUCAAGCUGUCU	6762-6780	2394	A-150941	AGACAGCUUGAGGUUUCAG	6762-6780	2674
AD-75218	A-150942	GUAUCAUGAAAAUGUCUAU	6806-6824	2395	A-150943	AUAGACAUUUUCAUGAUAC	6806-6824	2675
AD-75219	A-150944	UUCAAAAUAUCAAAACCUU	6824-6842	2396	A-150945	AAGGUUUUGAUAUUUUGAA	6824-6842	2676
AD-75220	A-150946	UUUCAAAUAUCACGCAGCU	6841-6859	2397	A-150947	AGCUGCGUGAUAUUUUGAAA	6841-6859	2677
AD-75221	A-150948	CUUAUAUUCAGUUUACAUA	6858-6876	2398	A-150949	UAUGUAAACUGAAUAUAAG	6858-6876	2678
AD-75222	A-150950	UUACAUAAAGGCCCCAAAU	6870-6888	2399	A-150951	AUUUGGGGCCUUUAUGUAA	6870-6888	2679
AD-75223	A-150952	AUACCAUGUCAGAUCUUUU	6887-6905	2400	A-150953	AAAAGAUCUGACAUGGUAU	6887-6905	2680
AD-75224	A-150954	AAAAGAGUUAAUGAACUAU	6910-6928	2401	A-150955	AUAGUUCAUUAACUCUUUU	6910-6928	2681
AD-75225	A-150956	AUGAGAAUUGGGAUUACAU	6927-6945	2402	A-150957	AUGUAAUCCCAAUUCUCAU	6927-6945	2682
AD-75226	A-150958	AUCAUGUAUUUUGCCUCAU	6944-6962	2403	A-150959	AUGAGGCAAAAUACAUGAU	6944-6962	2683
AD-75227	A-150960	UUAUCACACUUAUAGGCCA	6969-6987	2404	A-150961	UGGCCUAUAAGUGUGAUAA	6969-6987	2684
AD-75228	A-150962	CAAGUGUGAUAAAUAAACU	6986-7004	2405	A-150963	AGUUUAUUUAUCACACUUG	6986-7004	2685
AD-75229	A-150964	UUACAGACACUGAAUUAAU	7004-7022	2406	A-150965	AUUAAUUCAGUGUCUGUAA	7004-7022	2686
AD-75230	A-150966	UUUGAAACCAGAAAAUAAU	7035-7053	2407	A-150967	AUUAUUUUCUGGUUUCAAA	7035-7053	2687

Duplex Name	Sense Oligo Name	Sense Sequence	Position in NM_ 000618.3	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	Position in NM_ 000618.3	SEQ ID NO
AD-75231	A-150968	AUGACUGGCCAUUCGUUAA	7052-7070	2408	A-150969	UUAACGAAUGGCCAGUCAU	7052-7070	2688
AD-75232	A-150970	UUAGUUGAAAAGCAUAUUU	7078-7096	2409	A-150971	AAAUAUGCUUUUCAACUAA	7078-7096	2689
AD-75233	A-150972	UUUUUAUUAAAUUAAUUCU	7095-7113	2410	A-150973	AGAAUUAAUUUAAUAAAAA	7095-7113	2690
AD-75234	A-150974	CUGAUUGUAUUUGAAAUUA	7112-7130	2411	A-150975	UAAUUUCAAAUACAAUCAG	7112-7130	2691
AD-75235	A-150976	UUUGAAAUUAUUAUUCAAU	7121-7139	2412	A-150977	AUUGAAUAAUAAUUUCAAA	7121-7139	2692
AD-75236	A-150978	UUAUGGCAGAGGAAUAUCA	7144-7162	2413	A-150979	UGAUAUUCCUCUGCCAUAA	7144-7162	2693
AD-75237	A-150980	UCUAAAAAAUGUAACUAAUU	7175-7193	2414	A-150981	AAUUAGUUACAUUUUUAGA	7175-7193	2694
AD-75238	A-150982	UUUACUGUUUAAUAAGCAU	7210-7228	2415	A-150983	AUGCUUAUUAAACAGUAAA	7210-7228	2695
AD-75239	A-150984	UGUCAUAAUAAAAUGGUAU	7252-7270	2416	A-150985	AUACCAUUUUAUUAUGACA	7252-7270	2696
AD-75240	A-150986	AUAUCUUUCUUUAGUAAUU	7269-7287	2417	A-150987	AAUUACUAAAGAAAGAUAU	7269-7287	2697
AD-75241	A-150988	UUAGUAAUUACAUUAAAAU	7279-7297	2418	A-150989	AUUUUAAUGUAAUUACUAA	7279-7297	2698
AD-75242	A-150990	AUUAGUCAUGUUUGAUUAA	7296-7314	2419	A-150991	UUAAUCAAACAUGACUAAU	7296-7314	2699

Table 19. IGF-1 in vitro 10nM screen

Duplex Name	10nM AVG	10nM STD	Position in NM_000618.3
AD-74963	2.44	2.3	6-24
AD-74964	7.2	5.49	24-42
AD-74965	3.3	3.72	41-59
AD-74966	4.25	1.91	54-72
AD-74967	15.72	4.3	72-90
AD-74968	3.11	0.38	127-145
AD-74969	17.28	0.98	185-203
AD-74970	7.75	1.22	203-221
AD-74971	4.93	4	220-238
AD-74972	21.83	3.83	247-265
AD-74973	14.71	5.65	277-295
AD-74974	38.48	1.73	430-448
AD-74975	8.99	1.93	447-465
AD-74976	22.76	2.57	462-480
AD-74977	34.47	2.7	543-561
AD-74978	10.33	10.14	654-672
AD-74979	4.03	0.6	672-690
AD-74980	22.84	18.43	750-768
AD-74981	10.09	7.19	774-792
AD-74982	5.27	0.53	792-810
AD-74983	7.33	3.35	818-836
AD-74984	25.15	1.05	835-853
AD-74985	9.51	1.12	852-870
AD-74986	13.08	1.24	894-912
AD-74987	15.81	0.07	912-930
AD-74988	74.25	9.8	930-948
AD-74989	53.17	16.23	947-965
AD-74990	34.92	1.88	1091-1109
AD-74991	35.6	2.75	1108-1126
AD-74992	54.21	4.47	1125-1143
AD-74993	51.57	3.65	1135-1153
AD-74994	20.06	0.5	1144-1162
AD-74995	49.73	0.85	1162-1180
AD-74996	54.67	2.95	1195-1213

Duplex Name	10nM AVG	10nM STD	Position in NM_000618.3
AD-74997	24.76	9.85	1197-1215
AD-74998	116.31	7.45	1215-1233
AD-74999	37.97	1.63	1232-1250
AD-75000	17.29	1.27	1293-1311
AD-75001	44.75	3.5	1311-1329
AD-75002	33.61	1.4	1334-1352
AD-75003	50.72	3.07	1352-1370
AD-75004	49.47	3.37	1370-1388
AD-75005	33.73	0.68	1388-1406
AD-75006	62.64	3.34	1406-1424
AD-75007	36.36	0.24	1423-1441
AD-75008	37.81	2.85	1440-1458
AD-75009	19.35	1.31	1472-1490
AD-75010	71.78	3.37	1494-1512
AD-75011	25.82	2.55	1511-1529
AD-75012	55.66	5.16	1528-1546
AD-75013	83.97	4.37	1572-1590
AD-75014	29.26	11.24	1599-1617
AD-75015	40.19	0.14	1625-1643
AD-75016	38.98	4.61	1643-1661
AD-75017	32.96	4.54	1690-1708
AD-75018	19.3	1.74	1709-1727
AD-75019	71.36	5.08	1757-1775
AD-75020	20.46	1.94	1793-1811
AD-75021	33.72	1.42	1807-1825
AD-75022	84.23	3.34	1825-1843
AD-75023	24.26	1.03	1843-1861
AD-75024	40.83	2.06	1966-1984
AD-75025	48.65	1.28	2016-2034
AD-75026	70.35	5.83	2033-2051
AD-75027	94.74	14.09	2040-2058
AD-75028	29.93	7.99	2057-2075
AD-75029	31.95	1.57	2090-2108
AD-75030	64.72	8.6	2140-2158
AD-75031	44.8	3.93	2170-2188

Duplex Name	10nM AVG	10nM STD	Position in NM_000618.3
AD-75032	32.33	2.6	2192-2210
AD-75033	26.13	0.64	2210-2228
AD-75034	43.7	7.02	2228-2246
AD-75035	70.89	7.82	2249-2267
AD-75036	106.4	4.66	2266-2284
AD-75037	47.27	4.85	2285-2303
AD-75038	48.86	4.15	2303-2321
AD-75039	40.63	4.83	2322-2340
AD-75040	107.1	7.22	2340-2358
AD-75041	35.81	5.21	2357-2375
AD-75042	52.59	6.79	2398-2416
AD-75043	55.9	9.3	2432-2450
AD-75044	46.66	11.14	2449-2467
AD-75045	82.13	13.7	2453-2471
AD-75046	73.55	5.15	2470-2488
AD-75047	71.67	15.96	2482-2500
AD-75048	38.18	30.65	2521-2539
AD-75049	92.97	13.14	2539-2557
AD-75050	86	15.13	2548-2566
AD-75051	59.23	8.69	2566-2584
AD-75052	104.39	19.17	2584-2602
AD-75053	58.66	10.73	2601-2619
AD-75054	89.3	16.07	2618-2636
AD-75055	64.45	10.53	2623-2641
AD-75056	95.7	23.97	2640-2658
AD-75057	38.59	5.32	2648-2666
AD-75058	35.56	5.16	2666-2684
AD-75059	62.95	9.3	2684-2702
AD-75060	50.41	11.52	2771-2789
AD-75061	34.92	9.92	2793-2811
AD-75062	52.01	10.49	2811-2829
AD-75063	49.98	9.93	2828-2846
AD-75064	113.1	26.07	2869-2887
AD-75065	73.65	7.55	2906-2924
AD-75066	43.56	5.6	2920-2938

Duplex Name	10nM AVG	10nM STD	Position in NM_000618.3
AD-75067	56.32	9.44	2938-2956
AD-75068	57.61	8.64	2956-2974
AD-75069	34.69	12.05	2962-2980
AD-75070	89.29	14.15	2980-2998
AD-75071	64.02	16.69	3006-3024
AD-75072	125.6	49.27	3025-3043
AD-75073	111.64	19.51	3043-3061
AD-75074	75.61	13.18	3060-3078
AD-75075	111.51	16.74	3077-3095
AD-75076	69.43	9.4	3100-3118
AD-75077	44.04	5	3137-3155
AD-75078	57.27	10.2	3155-3173
AD-75079	28.28	5.64	3208-3226
AD-75080	59.53	7.5	3225-3243
AD-75081	61.41	5.87	3234-3252
AD-75082	54.31	7.14	3252-3270
AD-75083	34.99	5.51	3269-3287
AD-75084	46.86	9.9	3277-3295
AD-75085	56.82	7.96	3294-3312
AD-75086	58.83	11.06	3334-3352
AD-75087	55.26	2.93	3352-3370
AD-75088	34.12	16.28	3369-3387
AD-75089	63.74	11.09	3400-3418
AD-75090	36.81	7.73	3417-3435
AD-75091	31.56	5.56	3449-3467
AD-75092	61.39	11.47	3517-3535
AD-75093	83.2	19.37	3535-3553
AD-75094	80.16	13.64	3567-3585
AD-75095	38.33	8.26	3585-3603
AD-75096	103.57	22.84	3603-3621
AD-75097	69.98	7.03	3621-3639
AD-75098	51.57	14.6	3662-3680
AD-75099	31.97	13.18	3686-3704
AD-75100	94.94	9.26	3704-3722
AD-75101	36.43	11.54	3721-3739

Duplex Name	10nM AVG	10nM STD	Position in NM_000618.3
AD-75102	70.66	7.75	3732-3750
AD-75103	57.38	5.65	3750-3768
AD-75104	77.58	15.39	3767-3785
AD-75105	70.13	8.3	3770-3788
AD-75106	50.24	6.25	3788-3806
AD-75107	34.8	6.73	3806-3824
AD-75108	58.82	3.73	3835-3853
AD-75109	65.08	10.73	3865-3883
AD-75110	31.63	14.97	3939-3957
AD-75111	5.82	0.91	3982-4000
AD-75112	11.18	0.76	4000-4018
AD-75113	38.66	8.55	4081-4099
AD-75114	14.58	5.96	4098-4116
AD-75115	12.98	2.49	4101-4119
AD-75116	35.3	6.1	4119-4137
AD-75117	57.1	9.56	4137-4155
AD-75118	23.23	4.43	4154-4172
AD-75119	54.12	7.09	4208-4226
AD-75120	15.15	2.22	4271-4289
AD-75121	19.41	4.81	4289-4307
AD-75122	33.51	8.79	4319-4337
AD-75123	10.61	1.98	4336-4354
AD-75124	22.01	6.31	4341-4359
AD-75125	10.88	0.88	4359-4377
AD-75126	84.91	10.8	4377-4395
AD-75127	71.33	14.6	4395-4413
AD-75128	78.44	5.21	4412-4430
AD-75129	76.77	25.96	4426-4444
AD-75130	15.56	6.25	4443-4461
AD-75131	13.16	4.18	4460-4478
AD-75132	86.74	17.91	4464-4482
AD-75133	36.15	4.26	4484-4502
AD-75134	51.96	9.57	4511-4529
AD-75135	19.14	4.52	4566-4584
AD-75136	43.64	6.37	4584-4602

Duplex Name	10nM AVG	10nM STD	Position in NM_000618.3
AD-75137	3.8	0.43	4601-4619
AD-75138	40.31	6.39	4625-4643
AD-75139	20.1	4.15	4642-4660
AD-75140	53.32	1.96	4645-4663
AD-75141	51.87	3.72	4669-4687
AD-75142	32.17	4.32	4686-4704
AD-75143	17.5	6.52	4699-4717
AD-75144	94.26	24.89	4717-4735
AD-75145	8.73	1.94	4734-4752
AD-75146	39.8	9.22	4751-4769
AD-75147	44.81	9.36	4770-4788
AD-75148	21.99	3.67	4780-4798
AD-75149	56.19	8.99	4798-4816
AD-75150	22.7	2.67	4815-4833
AD-75151	33.03	2.82	4825-4843
AD-75152	28.29	11.66	4884-4902
AD-75153	34.14	6.63	4911-4929
AD-75154	43.03	6.81	4987-5005
AD-75155	10.11	2.68	5004-5022
AD-75156	49.77	4.33	5016-5034
AD-75157	10.55	1.54	5050-5068
AD-75158	98.46	15.78	5098-5116
AD-75159	59.88	7.14	5115-5133
AD-75160	67.78	10.53	5127-5145
AD-75161	71.58	12.59	5153-5171
AD-75162	9.5	2.65	5171-5189
AD-75163	99.59	10.77	5189-5207
AD-75164	24.32	6.23	5206-5224
AD-75165	48.65	10.6	5223-5241
AD-75166	20.31	3.83	5238-5256
AD-75167	96.76	8.31	5284-5302
AD-75168	50.33	6.42	5301-5319
AD-75169	35.41	3.61	5311-5329
AD-75170	9.16	1.79	5328-5346
AD-75171	74.55	6.92	5383-5401

Duplex Name	10nM AVG	10nM STD	Position in NM_000618.3
AD-75172	61.38	10.44	5391-5409
AD-75173	18.61	2.81	5409-5427
AD-75174	12.46	3.74	5412-5430
AD-75175	64.04	8.99	5430-5448
AD-75176	47.5	11.54	5505-5523
AD-75177	26.63	1.26	5570-5588
AD-75178	93.5	13.15	5603-5621
AD-75179	13.82	1.72	5620-5638
AD-75180	40.37	3.65	5628-5646
AD-75181	68.1	14	5645-5663
AD-75182	91.45	7.76	5659-5677
AD-75183	52.7	9.56	5677-5695
AD-75184	10.64	1.91	5694-5712
AD-75185	22.43	3.75	5715-5733
AD-75186	39.19	4.23	5740-5758
AD-75187	80.34	23.05	5773-5791
AD-75188	18.72	4.87	5790-5808
AD-75189	71.77	15.33	5884-5902
AD-75190	69.3	7.75	5917-5935
AD-75191	13.99	6.28	5934-5952
AD-75192	83.17	9.31	5942-5960
AD-75193	79.66	18.37	5959-5977
AD-75194	64.7	6.93	6066-6084
AD-75195	79.21	5.63	6129-6147
AD-75196	105.5	9.43	6147-6165
AD-75197	128.21	12.85	6164-6182
AD-75198	46.08	7.51	6231-6249
AD-75199	117.2	7.51	6303-6321
AD-75200	50.47	12.5	6346-6364
AD-75201	59.91	14.09	6364-6382
AD-75202	107.53	22.57	6382-6400
AD-75203	25.97	4.96	6399-6417
AD-75204	71.96	3.58	6444-6462
AD-75205	39.19	13.83	6461-6479
AD-75206	11.68	4.11	6479-6497

Duplex Name	10nM AVG	10nM STD	Position in NM_000618.3
AD-75207	40.79	10.66	6497-6515
AD-75208	34.43	5.15	6517-6535
AD-75209	107.98	25.75	6538-6556
AD-75210	52.4	6.95	6557-6575
AD-75211	90.01	19.17	6576-6594
AD-75212	22.91	6.75	6594-6612
AD-75213	87.12	9.51	6612-6630
AD-75214	19.83	5.95	6629-6647
AD-75215	50.88	6.82	6653-6671
AD-75216	43.88	4.7	6722-6740
AD-75217	18.19	2.83	6762-6780
AD-75218	9.91	1.3	6806-6824
AD-75219	54.72	5.78	6824-6842
AD-75220	60.73	10.07	6841-6859
AD-75221	23.15	1.19	6858-6876
AD-75222	36.29	4.92	6870-6888
AD-75223	21.88	2.24	6887-6905
AD-75224	32.13	3.75	6910-6928
AD-75225	12.65	3.49	6927-6945
AD-75226	48.19	14.5	6944-6962
AD-75227	58.51	8.21	6969-6987
AD-75228	53.16	6.09	6986-7004
AD-75229	10.94	2.92	7004-7022
AD-75230	31.83	5.18	7035-7053
AD-75231	15.75	2.98	7052-7070
AD-75232	18.71	3.47	7078-7096
AD-75233	106.56	8.46	7095-7113
AD-75234	18.49	4.54	7112-7130
AD-75235	113.68	22.34	7121-7139
AD-75236	20.92	10.52	7144-7162
AD-75237	29.1	4.1	7175-7193
AD-75238	39.59	10.16	7210-7228
AD-75239	23.08	3.81	7252-7270
AD-75240	98.59	16.79	7269-7287

Duplex Name	10nM AVG	10nM STD	Position in NM_000618.3
AD-75241	109.49	8.89	7279-7297
AD-75242	92.6	7.78	7296-7314

Table 20. Modified Sense and Antisense Strand Sequences of IGF-1 dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-74963	A-150432	UAGAUAAAUGUGAGGAUUUdTdT	2700	A-150433	AAAUCCUCACAUUUAUCUAdTdT	2980	UAGAUAAAUGUGAGGAUUU	3260
AD-74964	A-150434	UUCUCUAAAUCCCUCUUCUdTdT	2701	A-150435	AGAAGAGGGAUUUAGAGAAdTdT	2981	UUCUCUAAAUCCCUCUUCU	3261
AD-74965	A-150436	CUGUUUGCUAAAUCUCACUdTdT	2702	A-150437	AGUGAGAUUUAGCAAACAGdTdT	2982	CUGUUUGCUAAAUCUCACU	3262
AD-74966	A-150438	CUCACUGUCACUGCUAAAUdTdT	2703	A-150439	AUUUAGCAGUGACAGUGAGdTdT	2983	CUCACUGUCACUGCUAAAU	3263
AD-74967	A-150440	UUCAGAGCAGAUAGAGCCUdTdT	2704	A-150441	AGGCUCUAUCUGCUCUGAAdTdT	2984	UUCAGAGCAGAUAGAGCCU	3264
AD-74968	A-150442	CAUUGCUCUCAACAUCUCAdTdT	2705	A-150443	UGAGAUGUUGAGAGCAAUGdTdT	2985	CAUUGCUCUCAACAUCUCC	3265
AD-74969	A-150444	ACCAAUUCAUUUUCAGACUdTdT	2706	A-150445	AGUCUGAAAAUGAAUUGGUdTdT	2986	ACCAAUUCAUUUUCAGACU	3266
AD-74970	A-150446	UUUGUACUUCAGAAGCAAUdTdT	2707	A-150447	AUUGCUUCUGAAGUACAAAdTdT	2987	UUUGUACUUCAGAAGCAAU	3267
AD-74971	A-150448	AUGGGAAAAAUCAGCAGUAdTdT	2708	A-150449	UACUGCUGAUUUUUCCCAUdTdT	2988	AUGGGAAAAAUCAGCAGUC	3268
AD-74972	A-150450	CAAUUAUUUAAGUGCUGCUdTdT	2709	A-150451	AGCAGCACUUAAAUAAUUGdTdT	2989	CAAUUAUUUAAGUGCUGCU	3269
AD-74973	A-150452	UUGAAGGUGAAGAUGCACAdTdT	2710	A-150453	UGUGCAUCUUCACCUUCAAdTdT	2990	UUGAAGGUGAAGAUGCACA	3270
AD-74974	A-150454	UUUUAUUUCAACAAGCCCAdTdT	2711	A-150455	UGGGCUUGUUGAAAUAAAAdTdT	2991	UUUUAUUUCAACAAGCCCA	3271
AD-74975	A-150456	CACAGGGUAUGGCUCCAGAdTdT	2712	A-150457	UCUGGAGCCAUACCCUGUGdTdT	2992	CACAGGGUAUGGCUCCAGC	3272
AD-74976	A-150458	CAGCAGUCGGAGGGCGCCUdTdT	2713	A-150459	AGGCGCCCUCCGACUGCUGdTdT	2993	CAGCAGUCGGAGGGCGCCU	3273
AD-74977	A-150460	UUGCGCACCCCUCAAGCCUdTdT	2714	A-150461	AGGCUUGAGGGGUGCGCAAdTdT	2994	UUGCGCACCCCUCAAGCCU	3274
AD-74978	A-150462	UGCAGGAAACAAGAACUAAdTdT	2715	A-150463	UUAGUUCUUGUUUCCUGCAdTdT	2995	UGCAGGAAACAAGAACUAC	3275
AD-74979	A-150464	CAGGAUGUAGGAAGACCCUdTdT	2716	A-150465	AGGGUCUUCCUACAUCCUGdTdT	2996	CAGGAUGUAGGAAGACCCU	3276
AD-74980	A-150466	UUAAACUUUGGAACACCUAdTdT	2717	A-150467	UAGGUGUUCCAAAGUUUAAdTdT	2997	UUAAACUUUGGAACACCUA	3277
AD-74981	A-150468	AAAUAAGUUUGAUAACAUUdTdT	2718	A-150469	AAUGUUAUCAAACUUAUUUdTdT	2998	AAAUAAGUUUGAUAACAUU	3278
AD-74982	A-150470	UUAAAAGAUGGGCGUUUCAdTdT	2719	A-150471	UGAAACGCCCAUCUUUUAAdTdT	2999	UUAAAAGAUGGGCGUUUCC	3279
AD-74983	A-150472	AAAUACACAAGUAAACAUUdTdT	2720	A-150473	AAUGUUUACUUGUGUAUUUdTdT	3000	AAAUACACAAGUAAACAUU	3280
AD-74984	A-150474	UUCCAACAUUGUCUUUAGAdTdT	2721	A-150475	UCUAAAGACAAUGUUGGAAdTdT	3001	UUCCAACAUUGUCUUUAGG	3281
AD-74985	A-150476	GGAGUGAUUUGCACCUUGAdTdT	2722	A-150477	UCAAGGUGCAAAUCACUCCdTdT	3002	GGAGUGAUUUGCACCUUGC	3282
AD-74986	A-150478	AUUGCUGUUGAUCUUUUAUdTdT	2723	A-150479	AUAAAAGAUCAACAGCAAUdTdT	3003	AUUGCUGUUGAUCUUUUAU	3283
AD-74987	A-150480	UCAAUAAUGUUCUAUAGAAdTdT	2724	A-150481	UUCUAUAGAACAUUAUUGAdTdT	3004	UCAAUAAUGUUCUAUAGAA	3284

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-74988	A-150482	AAAGAAAAAAAAAAAAUAUAUdTdT	2725	A-150483	AUAUAUUUUUUUUUUCUUUdTdT	3005	AAAGAAAAAAAAAAAUAUAU	3285
AD-74989	A-150484	AUAUAUAUAUAUAUAUCUUAAdTdT	2726	A-150485	UUAAGAUAUAUAUAUAUAUdTdT	3006	AUAUAUAUAUAUAUCUUAG	3286
AD-74990	A-150486	UUUCCUUAUUUGCACUUCUdTdT	2727	A-150487	AGAAGUGCAAAUAAGGAAAdTdT	3007	UUUCCUUAUUUGCACUUCU	3287
AD-74991	A-150488	CUUUCUACACAACUCGGAdTdT	2728	A-150489	UCCCGAGUUGUGUAGAAAGdTdT	3008	CUUUCUACACAACUCGGGC	3288
AD-74992	A-150490	GCUGUUUGUUUUACAGUGUdTdT	2729	A-150491	ACACUGUAAAACAAACAGCdTdT	3009	GCUGUUUGUUUUACAGUGU	3289
AD-74993	A-150492	UUACAGUGUCUGAUAAUCUdTdT	2730	A-150493	AGAUUAUCAGACACUGUAAdTdT	3010	UUACAGUGUCUGAUAAUCU	3290
AD-74994	A-150494	CUGAUAAUCUUGUUAGUCUdTdT	2731	A-150495	AGACUAACAAGAUUAUCAGdTdT	3011	CUGAUAAUCUUGUUAGUCU	3291
AD-74995	A-150496	UAUACCCACCACCUCCCUUdTdT	2732	A-150497	AAGGGAGGUGGGUGGGUAUAdTdT	3012	UAUACCCACCACCUCCCUU	3292
AD-74996	A-150498	UUGCCGAAUUUGGCCUCCUdTdT	2733	A-150499	AGGAGGCCAAAUUCGGCAAdTdT	3013	UUGCCGAAUUUGGCCUCCU	3293
AD-74997	A-150500	GCCGAAUUUGGCCUCCUCAdTdT	2734	A-150501	UGAGGAGGCCAAAUUCGGCdTdT	3014	GCCGAAUUUGGCCUCCUCA	3294
AD-74998	A-150502	AAAAGCAGCAGCAAGUCGUdTdT	2735	A-150503	ACGACUUGCUGCUGCUUUUdTdT	3015	AAAAGCAGCAGCAAGUCGU	3295
AD-74999	A-150504	GUCAAGAAGCACACCAAUUdTdT	2736	A-150505	AAUUGGUGUGCUUCUUGACdTdT	3016	GUCAAGAAGCACACCAAUU	3296
AD-75000	A-150506	AGUUGGAUGCAUUUUAUUUUdTdT	2737	A-150507	AAAUAAAAAUGCAUCCAACUdTdT	3017	AGUUGGAUGCAUUUUAUUU	3297
AD-75001	A-150508	UUAGACACAAAGCUUUAUUdTdT	2738	A-150509	AAUAAAGCUUUGUCUAAAdTdT	3018	UUAGACACAAAGCUUUAUU	3298
AD-75002	A-150510	CACAUCAUGCUUACAAAAAAdTdT	2739	A-150511	UUUUUGUAAGCAUGAUGUGdTdT	3019	CACAUCAUGCUUACAAAAA	3299
AD-75003	A-150512	AAGAAUAAUGCAAAUAGUUdTdT	2740	A-150513	AACUAUUUGCAUUAUUCUUdTdT	3020	AAGAAUAAUGCAAAUAGUU	3300
AD-75004	A-150514	UGCAACUUUGAGGCCAAUAdTdT	2741	A-150515	UAUUGGCCUCAAAGUUGCAdTdT	3021	UGCAACUUUGAGGCCAAUC	3301
AD-75005	A-150516	CAUUUUUAGGCAUAUGUUUdTdT	2742	A-150517	AAACAUAUGCCUAAAAAUGdTdT	3022	CAUUUUUAGGCAUAUGUUU	3302
AD-75006	A-150518	UUAAACAUAGAAAGUUUCUdTdT	2743	A-150519	AGAAACUUUCUAUGUUUAAdTdT	3023	UUAAACAUAGAAAGUUUCU	3303
AD-75007	A-150520	CUUCAACUCAAAAGAGUUAdTdT	2744	A-150521	UAACUCUUUUGAGUUGAAGdTdT	3024	CUUCAACUCAAAAGAGUUC	3304
AD-75008	A-150522	UCCUUCAAAUGAUGAGUUAdTdT	2745	A-150523	UAACUCAUCAUUUGAAGGAdTdT	3025	UCCUUCAAAUGAUGAGUUA	3305
AD-75009	A-150524	UUAGUAACUUUCCUCUUUUdTdT	2746	A-150525	AAAAGAGGAAAGUUACUAAdTdT	3026	UUAGUAACUUUCCUCUUUU	3306
AD-75010	A-150526	UUUUUCCAUAUAGAGCACUdTdT	2747	A-150527	AGUGCUCUAUAUGGAAAAAdTdT	3027	UUUUUCCAUAUAGAGCACU	3307
AD-75011	A-150528	CUAUGUAAAAUUUAGCAUAUdTdT	2748	A-150529	AUAUGCUAAAAUUUACAUAGdTdT	3028	CUAUGUAAAAUUUAGCAUAU	3308
AD-75012	A-150530	AUCAAUUAUACAGGAUAUAdTdT	2749	A-150531	UAUAUCCUGUAUAAUUGAUdTdT	3029	AUCAAUUAUACAGGAUAUA	3309
AD-75013	A-150532	UUUAGUAUAAAUGGUGCUAUdTdT	2750	A-150533	AUAGCACCAUUAUACUAAAdTdT	3030	UUUAGUAUAAAUGGUGCUAU	3310
AD-75014	A-150534	UUGUUAUAUGAAAGAGUCUdTdT	2751	A-150535	AGACUCUUUCAUAUAACAAdTdT	3031	UUGUUAUAUGAAAGAGUCU	3311

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-75015	A-150536	ACGGUAAUACGUGAAAGCAdTdT	2752	A-150537	UGCUUUCACGUAUUACCGUdTdT	3032	ACGGUAAUACGUGAAAGCA	3312
AD-75016	A-150538	AAACACAAUAGGGGGAAGCCUdTdT	2753	A-150539	AGGCUUCCCCUAUUGUUUUdTdT	3033	AAACACAAUAGGGGAAGCCU	3313
AD-75017	A-150540	UACUGAAAAACACCAUCCAUdTdT	2754	A-150541	AUGGAUGGUGUUUUCAGUAdTdT	3034	UACUGAAAAACACCAUCCAU	3314
AD-75018	A-150542	UUGGGAAAGAAGGCAAAGUdTdT	2755	A-150543	ACUUUGCCUUCUUUCCCAAdTdT	3035	UUGGGAAAGAAGGCAAAGU	3315
AD-75019	A-150544	UCAGACACAAAAGUCCACUdTdT	2756	A-150545	AGUGGACUUUUGUGUCUGAdTdT	3036	UCAGACACAAAAGUCCACU	3316
AD-75020	A-150546	CGAGUCCAGAGAGGAAACUdTdT	2757	A-150547	AGUUUCCUCUCUGGACUCGdTdT	3037	CGAGUCCAGAGAGGAAACU	3317
AD-75021	A-150548	AAACUGUGGAAUGGAAAAAdTdT	2758	A-150549	UUUUUCCAUUCCACAGUUUdTdT	3038	AAACUGUGGAAUGGAAAAA	3318
AD-75022	A-150550	AGCAGAAGGCUAGGAAUUUdTdT	2759	A-150551	AAAUUCCUAGCCUUCUGCUdTdT	3039	AGCAGAAGGCUAGGAAUUU	3319
AD-75023	A-150552	UUAGCAGUCCUGGUUUCUUdTdT	2760	A-150553	AAGAAACCAGGACUGCUAAdTdT	3040	UUAGCAGUCCUGGUUUCUU	3320
AD-75024	A-150554	CAAAAUGGGGGCAAUAUGUdTdT	2761	A-150555	ACAUAUUGCCCCCAUUUUGdTdT	3041	CAAAAUGGGGGCAAUAUGU	3321
AD-75025	A-150556	UUUAAAAAGAUAAAGAUUAdTdT	2762	A-150557	UAAUCUUUAUCUUUUUUAAAdTdT	3042	UUUAAAAAGAUAAAGAUUAC	3322
AD-75026	A-150558	UCAGAUUUUUUUUACCCUAdTdT	2763	A-150559	UAGGGUAAAAAAAAAUCUGAdTdT	3043	UCAGAUUUUUUUUACCCUG	3323
AD-75027	A-150560	UUUUUUACCCUGGGUUGCUdTdT	2764	A-150561	AGCAACCCAGGGUAAAAAAAdTdT	3044	UUUUUUACCCUGGGUUGCU	3324
AD-75028	A-150562	CUGUAAGGGUGCAACAUCAdTdT	2765	A-150563	UGAUGUUGCACCCUUACAGdTdT	3045	CUGUAAGGGUGCAACAUCA	3325
AD-75029	A-150564	CUGAGAUGCAAGGAAUUCUdTdT	2766	A-150565	AGAAUUCCUUGCAUCUCAGdTdT	3046	CUGAGAUGCAAGGAAUUCU	3326
AD-75030	A-150566	UUGGUGAAUUGAAUGCUCAdTdT	2767	A-150567	UGAGCAUUCAAUUCACCAAdTdT	3047	UUGGUGAAUUGAAUGCUCC	3327
AD-75031	A-150568	UUCUUGUCAGUGAAGCUAUdTdT	2768	A-150569	AUAGCUUCACUGACAAGAAdTdT	3048	UUCUUGUCAGUGAAGCUAU	3328
AD-75032	A-150570	AAUAACUGGCCAACUAGUUdTdT	2769	A-150571	AACUAGUUGGCCAGUUAUUdTdT	3049	AAUAACUGGCCAACUAGUU	3329
AD-75033	A-150572	UGUUAAAAAGCUAACAGCUAdTdT	2770	A-150573	UAGCUGUUAGCUUUUAACAdTdT	3050	UGUUAAAAAGCUAACAGCUC	3330
AD-75034	A-150574	CAAUCUCUUAAAACACUUUdTdT	2771	A-150575	AAAGUGUUUUAAGAGAUUGdTdT	3051	CAAUCUCUUAAAACACUUU	3331
AD-75035	A-150576	AAAAUAUGUGGGAAGCAUUdTdT	2772	A-150577	AAUGCUUCCCACAUAUUUUdTdT	3052	AAAAUAUGUGGGAAGCAUU	3332
AD-75036	A-150578	UUUGAUUUUCAAUUUGAUUdTdT	2773	A-150579	AAUCAAAUUGAAAAUCAAAdTdT	3053	UUUGAUUUUCAAUUUGAUU	3333
AD-75037	A-150580	UUGAAUUCUGCAUUUGGUUdTdT	2774	A-150581	AACCAAAUGCAGAAUUCAAdTdT	3054	UUGAAUUCUGCAUUUGGUU	3334
AD-75038	A-150582	UUUAUGAAUACAAAGAUAAdTdT	2775	A-150583	UUAUCUUUGUAUUCAUAAAdTdT	3055	UUUAUGAAUACAAAGAUAA	3335
AD-75039	A-150584	GUGAAAAGAGAGAAAGGAAdTdT	2776	A-150585	UUCCUUUCUCUCUUUUCACdTdT	3056	GUGAAAAGAGAGAAAGGAA	3336
AD-75040	A-150586	AAAGAAAAAGGAGAAAAACdTdT	2777	A-150587	GUUUUUCUCCUUUUUCUUUdTdT	3057	AAAGAAAAAGGAGAAAAAC	3337
AD-75041	A-150588	ACAAAGAGAUUUCUACCAAdTdT	2778	A-150589	UUGGUAGAAAUCUCUUUGUdTdT	3058	ACAAAGAGAUUUCUACCAG	3338

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-75042	A-150590	UUGUUAGCACUCACUGACUdTdT	2779	A-150591	AGUCAGUGAGUGCUAACAAdTdT	3059	UUGUUAGCACUCACUGACU	3339
AD-75043	A-150592	UACAUAUCUAGUAAAAACCUdTdT	2780	A-150593	AGGUUUUACUAGAUAUGUAdTdT	3060	UACAUAUCUAGUAAAACCU	3340
AD-75044	A-150594	CUCGUUUAAUACUAUAAAUdTdT	2781	A-150595	AUUUAUAGUAUUAAACGAGdTdT	3061	CUCGUUUAAUACUAUAAAU	3341
AD-75045	A-150596	UUUAAUACUAUAAAAUAAUAdTdT	2782	A-150597	UAUUAUUUAUAGUAUUAAAdTdT	3062	UUUAAUACUAUAAAUAAUA	3342
AD-75046	A-150598	UAUUCUAUUCAUUUUGAAAdTdT	2783	A-150599	UUUCAAAAUGAAUAGAAUAdTdT	3063	UAUUCUAUUCAUUUUGAAA	3343
AD-75047	A-150600	UUUGAAAAACACAAUGAUUdTdT	2784	A-150601	AAUCAUUGUGUUUUUCAAAdTdT	3064	UUUGAAAAACACAAUGAUU	3344
AD-75048	A-150602	AAGGAAAGUGAUCCAAAAUUdTdT	2785	A-150603	AUUUUGGAUCACUUUCCUUdTdT	3065	AAGGAAAGUGAUCCAAAAU	3345
AD-75049	A-150604	UUUGAAAUAUUAAAUAUAAUdTdT	2786	A-150605	AUUAUUUUAAUAUUUCAAAdTdT	3066	UUUGAAAUAUUAAAUAAAU	3346
AD-75050	A-150606	UUAAAAUAAUAUCUAAUAAAdTdT	2787	A-150607	UUAUUAGAUAUUAUUUUAAdTdT	3067	UUAAAAUAAUAUCUAAUAA	3347
AD-75051	A-150608	AAAAGUCACAAAGUUAUCUdTdT	2788	A-150609	AGAUAACUUUGUGACUUUUdTdT	3068	AAAAGUCACAAAGUUAUCU	3348
AD-75052	A-150610	UUCUUUAACAAACUUUACUdTdT	2789	A-150611	AGUAAAGUUUGUUAAAGAAdTdT	3069	UUCUUUAACAAACUUUACU	3349
AD-75053	A-150612	CUCUUAUUCUUAGCUGUAUdTdT	2790	A-150613	AUACAGCUAAGAAUAAGAGdTdT	3070	CUCUUAUUCUUAGCUGUAU	3350
AD-75054	A-150614	AUAUACAUUUUUUUUAAAAGdTdT	2791	A-150615	CUUUUAAAAAAAAAUGUAUAUdTdT	3071	AUAUACAUUUUUUUUAAAAG	3351
AD-75055	A-150616	CAUUUUUUUAAAAGUUUGUdTdT	2792	A-150617	ACAAACUUUUAAAAAAAAAUGdTdT	3072	CAUUUUUUUAAAAGUUUGU	3352
AD-75056	A-150618	GUUAAAAUAUGCUUGACUAdTdT	2793	A-150619	UAGUCAAGCAUAUUUUAACdTdT	3073	GUUAAAAUAUGCUUGACUA	3353
AD-75057	A-150620	AUGCUUGACUAGAGUUUCAdTdT	2794	A-150621	UGAAACUCUAGUCAAGCAUdTdT	3074	AUGCUUGACUAGAGUUUCC	3354
AD-75058	A-150622	CAGUUGAAAGGCAAAAACUdTdT	2795	A-150623	AGUUUUUGCCUUUCAACUGdTdT	3075	CAGUUGAAAGGCAAAAACU	3355
AD-75059	A-150624	UUCCAUCACAACAAGAAAUdTdT	2796	A-150625	AUUUCUUGUUGUGAUGGAAdTdT	3076	UUCCAUCACAACAAGAAAU	3356
AD-75060	A-150626	UUGGUAUCAAGAAAGUCCAdTdT	2797	A-150627	UGGACUUUCUUGAUACCAAdTdT	3077	UUGGUAUCAAGAAAGUCCA	3357
AD-75061	A-150628	GUUAGUGUACUAGUCCAUAdTdT	2798	A-150629	UAUGGACUAGUACACUAACdTdT	3078	GUUAGUGUACUAGUCCAUC	3358
AD-75062	A-150630	CAUAGCCUAGAAAAUGAUAdTdT	2799	A-150631	UAUCAUUUUCUAGGCUAUGdTdT	3079	CAUAGCCUAGAAAAUGAUC	3359
AD-75063	A-150632	UCCCUAUCUGCAGAUCAAAdTdT	2800	A-150633	UUUGAUCUGCAGAUAGGGAdTdT	3080	UCCCUAUCUGCAGAUCAAG	3360
AD-75064	A-150634	UUAUCCAGCAUUCAGAUCUdTdT	2801	A-150635	AGAUCUGAAUGCUGGAUAAdTdT	3081	UUAUCCAGCAUUCAGAUCU	3361
AD-75065	A-150636	UUUUUGGUUAAAAGUACCAdTdT	2802	A-150637	UGGUACUUUUAACCAAAAAdTdT	3082	UUUUUGGUUAAAAGUACCC	3362
AD-75066	A-150638	UACCCAGGCUUGAUUAUUUdTdT	2803	A-150639	AAAUAAUCAAGCCUGGUAdTdT	3083	UACCCAGGCUUGAUUAUUU	3363
AD-75067	A-150640	UCAUGCAAAUUCUAUAUUUdTdT	2804	A-150641	AAAUAUAGAAUUUGCAUGAdTdT	3084	UCAUGCAAAUUCUAUAUUU	3364
AD-75068	A-150642	UUACAUUCUUGGAAAGUCUdTdT	2805	A-150643	AGACUUUCCAAGAAUGUAAdTdT	3085	UUACAUUCUUGGAAAGUCU	3365

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-75069	A-150644	UCUUGGAAAGUCUAUAUGAdTdT	2806	A-150645	UCAUAUAGACUUUCCAAGAdTdT	3086	UCUUGGAAAGUCUAUAUGA	3366
AD-75070	A-150646	AAAAACAAAAAUAACAUCUdTdT	2807	A-150647	AGAUGUUAUUUUUGUUUUUdTdT	3087	AAAAACAAAAAUAACAUCU	3367
AD-75071	A-150648	UUCUCCCACUGGGUCACCUdTdT	2808	A-150649	AGGUGACCCAGUGGGAGAAdTdT	3088	UUCUCCCACUGGGUCACCU	3368
AD-75072	A-150650	CAAGGAUCAGAGGCCAGGAdTdT	2809	A-150651	UCCUGGCCUCUGAUCCUUGdTdT	3089	CAAGGAUCAGAGGCCAGGA	3369
AD-75073	A-150652	AAAAAAAAAAAAAAGACUAdTdT	2810	A-150653	UAGUCUUUUUUUUUUUUUUdTdT	3090	AAAAAAAAAAAAAAGACUC	3370
AD-75074	A-150654	UCCCUGGAUCUCUGAAUAUdTdT	2811	A-150655	AUAUUCAGAGAUCCAGGGAdTdT	3091	UCCCUGGAUCUCUGAAUAU	3371
AD-75075	A-150656	AUAUGCAAAAAGAAGGCCAdTdT	2812	A-150657	UGGCCUUCUUUUUGCAUAUdTdT	3092	AUAUGCAAAAAGAAGGCCC	3372
AD-75076	A-150658	UAGUGGAGCCAGCAAUCCUdTdT	2813	A-150659	AGGAUUGCUGGCUCCACUAdTdT	3093	UAGUGGAGCCAGCAAUCCU	3373
AD-75077	A-150660	UUAACUCUCAGUCCAACACAUdTdT	2814	A-150661	AUGUUGGACUGAGAGUUAAdTdT	3094	UUAACUCUCAGUCCAACAU	3374
AD-75078	A-150662	UUAUUUGAAUUGAGCACCUdTdT	2815	A-150663	AGGUGCUCAAUUCAAAUAAdTdT	3095	UUAUUUGAAUUGAGCACCU	3375
AD-75079	A-150664	CAGAUGUAAAAGAAACUAUdTdT	2816	A-150665	AUAGUUUCUUUUACAUCUGdTdT	3096	CAGAUGUAAAAGAAACUAU	3376
AD-75080	A-150666	AUACAUCAUUUUUGCCCUAdTdT	2817	A-150667	UAGGGCAAAAAAUGAUGUAUdTdT	3097	AUACAUCAUUUUUGCCCUC	3377
AD-75081	A-150668	UUUUGCCCUCUGCCUGUUUdTdT	2818	A-150669	AAACAGGCAGAGGGCAAAAdTdT	3098	UUUUGCCCUCUGCCUGUUU	3378
AD-75082	A-150670	UUCCAGACAUACAGGUUCUdTdT	2819	A-150671	AGAACCUGUAUGUCUGGAAdTdT	3099	UUCCAGACAUACAGGUUCU	3379
AD-75083	A-150672	CUGUGGAAUAAGAUACUGAdTdT	2820	A-150673	UCAGUAUCUUAUUCCACAGdTdT	3100	CUGUGGAAUAAGAUACUGG	3380
AD-75084	A-150674	UAAGAUACUGGACUCCUCUdTdT	2821	A-150675	AGAGGAGUCCAGUAUCUUAdTdT	3101	UAAGAUACUGGACUCCUCU	3381
AD-75085	A-150676	CUUCCCAAGAUGGCACUUAdTdT	2822	A-150677	UAAGUGCCAUCUUGGGAAGdTdT	3102	CUUCCCAAGAUGGCACUUC	3382
AD-75086	A-150678	GUGUACCUUUUAAAAUUAUdTdT	2823	A-150679	AUAAUUUUAAAAGGUACACdTdT	3103	GUGUACCUUUUAAAAUUAU	3383
AD-75087	A-150680	UUCCCUCUCAACAAAACUUdTdT	2824	A-150681	AAGUUUUGUUGAGAGGGAAdTdT	3104	UUCCCUCUCAACAAAACUU	3384
AD-75088	A-150682	UUUAUAGGCAGUCUUCUGAdTdT	2825	A-150683	UCAGAAGACUGCCUAUAAAdTdT	3105	UUUAUAGGCAGUCUUCUGC	3385
AD-75089	A-150684	UUUUCUGUCAUAGUUAGAUdTdT	2826	A-150685	AUCUAACUAUGACAGAAAAdTdT	3106	UUUUCUGUCAUAGUUAGAU	3386
AD-75090	A-150686	AUGUGAUAAUUCUAAGAGUdTdT	2827	A-150687	ACUCUUAGAAUUAUCACAUdTdT	3107	AUGUGAUAAUUCUAAGAGU	3387
AD-75091	A-150688	UUCCUUCACUUAAUUCUAUdTdT	2828	A-150689	AUAGAAUUAAGUGAAGGAAdTdT	3108	UUCCUUCACUUAAUUCUAU	3388
AD-75092	A-150690	AUUAUCUUUCUUAACUUUUdTdT	2829	A-150691	AAAAGUUAAGAAAGAUAAUdTdT	3109	AUUAUCUUUCUUAACUUUU	3389
AD-75093	A-150692	UUCCAACACAUAAUCCUCUdTdT	2830	A-150693	AGAGGAUUAUGUGUUGGAAdTdT	3110	UUCCAACACAUAAUCCUCU	3390
AD-75094	A-150694	AAAUAAAUUGAAAAUAACUdTdT	2831	A-150695	AGUUAUUUUCAAUUUAUUUdTdT	3111	AAAUAAAUUGAAAAUAACU	3391
AD-75095	A-150696	UCAUUAUACCAAUUCACUAdTdT	2832	A-150697	UAGUGAAUUGGUAUAAUGAdTdT	3112	UCAUUAUACCAAUUCACUA	3392

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-75096	A-150698	AUUUUAUUUUUUAAUGAAUdTdT	2833	A-150699	AUUCAUUAAAAAAUAAAAUdTdT	3113	AUUUUAUUUUUUAAUGAAU	3393
AD-75097	A-150700	UUAAAACUAGAAAACAAAUdTdT	2834	A-150701	AUUUGUUUUCUAGUUUUAAdTdT	3114	UUAAAACUAGAAAACAAAU	3394
AD-75098	A-150702	UUGAUUACUAUAUACUACAdTdT	2835	A-150703	UGUAGUAUAUAGUAAUCAAdTdT	3115	UUGAUUACUAUAUACUACA	3395
AD-75099	A-150704	AUGACUCAGAUUUCAUAGAdTdT	2836	A-150705	UCUAUGAAAUCUGAGUCAUdTdT	3116	AUGACUCAGAUUUCAUAGA	3396
AD-75100	A-150706	AAAGGAGCAACCAAAAUGUdTdT	2837	A-150707	ACAUUUUGGUUGCUCCUUUdTdT	3117	AAAGGAGCAACCAAAAUGU	3397
AD-75101	A-150708	GUCACAACCCAAAACUUUAdTdT	2838	A-150709	UAAAGUUUUGGGUUGUGACdTdT	3118	GUCACAACCCAAAACUUUA	3398
AD-75102	A-150710	AAACUUUACAAGCUUUGCUdTdT	2839	A-150711	AGCAAAGCUUGUAAAGUUUdTdT	3119	AAACUUUACAAGCUUUGCU	3399
AD-75103	A-150712	UUCAGAAUUAGAUUGCUUUdTdT	2840	A-150713	AAAGCAAUCUAAUUCUGAAdTdT	3120	UUCAGAAUUAGAUUGCUUU	3400
AD-75104	A-150714	UUAUAAUUCUUGAAUGAGAdTdT	2841	A-150715	UCUCAUUCAAGAAUUAUAAdTdT	3121	UUAUAAUUCUUGAAUGAGG	3401
AD-75105	A-150716	UAAUUCUUGAAUGAGGCAAdTdT	2842	A-150717	UUGCCUCAUUCAAGAAUUAdTdT	3122	UAAUUCUUGAAUGAGGCAA	3402
AD-75106	A-150718	AUUUCAAGAUAUUUGUAAAdTdT	2843	A-150719	UUUACAAAUAUCUUGAAAUdTdT	3123	AUUUCAAGAUAUUUGUAAA	3403
AD-75107	A-150720	AAGAACAGUAAACAUUGGUdTdT	2844	A-150721	ACCAAUGUUUACUGUCUUUdTdT	3124	AAGAACAGUAAACAUUGGU	3404
AD-75108	A-150722	UUUCAACUCAUAGGCUUAUdTdT	2845	A-150723	AUAAGCCUAUGAGUUGAAAdTdT	3125	UUUCAACUCAUAGGCUUAU	3405
AD-75109	A-150724	UUGACCAUACUGGAUACUUdTdT	2846	A-150725	AAGUAUCCAGUAUGGUCAAdTdT	3126	UUGACCAUACUGGAUACUU	3406
AD-75110	A-150726	UUUAAGAUGAGGCAGUUCAdTdT	2847	A-150727	UGAACUGCCUCAUCUUAAAdTdT	3127	UUUAAGAUGAGGCAGUUCC	3407
AD-75111	A-150728	CAUCAGAAUCCACUCUUCUdTdT	2848	A-150729	AGAAGAGUGGAUUCUGAUGdTdT	3128	CAUCAGAAUCCACUCUUCU	3408
AD-75112	A-150730	UAGGGAUAUGAAAAUCUCUdTdT	2849	A-150731	AGAGAUUUUCAUAUCCCUAdTdT	3129	UAGGGAUAUGAAAAUCUCU	3409
AD-75113	A-150732	UUCACCCUAAGGAUCCAAUdTdT	2850	A-150733	AUUGGAUCCUUAGGGUGAAdTdT	3130	UUCACCCUAAGGAUCCAAU	3410
AD-75114	A-150734	AUGGAAUACUGAAAAGAAAdTdT	2851	A-150735	UUUCUUUUCAGUAUUCCAUdTdT	3131	AUGGAAUACUGAAAAGAAA	3411
AD-75115	A-150736	GAAUACUGAAAAGAAAUCAdTdT	2852	A-150737	UGAUUUCUUUUUCAGUAUUCdTdT	3132	GAAUACUGAAAAGAAAUCA	3412
AD-75116	A-150738	ACUUCCUUGAAAAUUUUAUdTdT	2853	A-150739	AUAAAAUUUUCAAGGAAGUdTdT	3133	ACUUCCUUGAAAAUUUUAU	3413
AD-75117	A-150740	UUAAAAAACAAACAAACAAdTdT	2854	A-150741	UUGUUUGUUUGUUUUUUAAdTdT	3134	UUAAAAAACAAACAAACAA	3414
AD-75118	A-150742	AAACAAAAAGCCUGUCCAAdTdT	2855	A-150743	UUGGACAGGCUUUUUGUUUUdTdT	3135	AAACAAAAAGCCUGUCCAC	3415
AD-75119	A-150744	UUUGUGUAGAUGAAACCAUdTdT	2856	A-150745	AUGGUUUCAUCUACACAAAdTdT	3136	UUUGUGUAGAUGAAACCAU	3416
AD-75120	A-150746	UUGGGAGAAGGCUUAGAAUdTdT	2857	A-150747	AUUCUAAGCCUUCUCCCAAdTdT	3137	UUGGGAGAAGGCUUAGAAU	3417
AD-75121	A-150748	UAAAAGAUGUAGCACAUUUdTdT	2858	A-150749	AAAUGUGCUACAUCUUUUAdTdT	3138	UAAAAGAUGUAGCACAUUU	3418
AD-75122	A-150750	UUAUUGUUUGGCCAGCUAUdTdT	2859	A-150751	AUAGCUGGCCAAACAAUAAdTdT	3139	UUAUUGUUUGGCCAGCUAU	3419

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-75123	A-150752	AUGCCAAUGUGGUGCUAUUdTdT	2860	A-150753	AAUAGCACCACAUUGGCAUdTdT	3140	AUGCCAAUGUGGUGCUAUU	3420
AD-75124	A-150754	AAUGUGGUGCUAUUGUUUAdTdT	2861	A-150755	UAAACAAUAGCACCACAUUdTdT	3141	AAUGUGGUGCUAUUGUUUC	3421
AD-75125	A-150756	CUUUAAGAAAGUACUUGAAdTdT	2862	A-150757	UUCAAGUACUUUCUUAAAGdTdT	3142	CUUUAAGAAAGUACUUGAC	3422
AD-75126	A-150758	CUAAAAAAAAAAAGAAAAAAdTdT	2863	A-150759	UUUUUUCUUUUUUUUUUAGdTdT	3143	CUAAAAAAAAAAAGAAAAAA	3423
AD-75127	A-150760	AAGAAAAAAAAAGAAAGCAUdTdT	2864	A-150761	AUGCUUUCUUUUUUUUCUUdTdT	3144	AAGAAAAAAAAAGAAAGCAU	3424
AD-75128	A-150762	AUAGACAUAUUUUUUUUAAAdTdT	2865	A-150763	UUUAAAAAAAAUAUGUCUAUdTdT	3145	AUAGACAUAUUUUUUUAAA	3425
AD-75129	A-150764	UUAAAGUAUAAAAAACAACAdTdT	2866	A-150765	UGUUGUUUUUAUACUUUAAdTdT	3146	UUAAAGUAUAAAAAACAACA	3426
AD-75130	A-150766	CAAUUCUAUAGAUAGAUGAdTdT	2867	A-150767	UCAUCUAUCUAUAGAAUUGdTdT	3147	CAAUUCUAUAGAUAGAUGG	3427
AD-75131	A-150768	GGCUUAAUAAAAAUAGCAUUdTdT	2868	A-150769	AAUGCUAUUUUAUUAAGCCdTdT	3148	GGCUUAAUAAAAAUAGCAUU	3428
AD-75132	A-150770	UAAUAAAAAUAGCAUUAGGUdTdT	2869	A-150771	ACCUAAUGCUAUUUUAUUAdTdT	3149	UAAUAAAAAUAGCAUUAGGU	3429
AD-75133	A-150772	UAUCUAGCCACCACCACCUdTdT	2870	A-150773	AGGUGGUGGUGGCUAGAUAdTdT	3150	UAUCUAGCCACCACCACCU	3430
AD-75134	A-150774	UUUAUCACUCACAAGUAGUdTdT	2871	A-150775	ACUACUUGUGAGUGAUAAAdTdT	3151	UUUAUCACUCACAAGUAGU	3431
AD-75135	A-150776	GGCAGGAGUUGGAAAUUUUUdTdT	2872	A-150777	AAAAUUUCCAACUCCUGCCdTdT	3152	GGCAGGAGUUGGAAAUUUU	3432
AD-75136	A-150778	UUUAAAGUUAGAAGGCUCAdTdT	2873	A-150779	UGAGCCUUCUAACUUUAAAdTdT	3153	UUUAAAGUUAGAAGGCUCC	3433
AD-75137	A-150780	CCAUUGUUUUGUUGGCUCUdTdT	2874	A-150781	AGAGCCAACAAAACAAUGGdTdT	3154	CCAUUGUUUUGUUGGCUCU	3434
AD-75138	A-150782	UUAGCAAAAUUAGCAAUAUdTdT	2875	A-150783	AUAUUGCUAAUUUUGCUAAdTdT	3155	UUAGCAAAAUUAGCAAUAU	3435
AD-75139	A-150784	AUAUUAUCCAAUCUUCUGAdTdT	2876	A-150785	UCAGAAGAUUGGAUAAUAUdTdT	3156	AUAUUAUCCAAUCUUCUGA	3436
AD-75140	A-150786	UUAUCCAAUCUUCUGAACUdTdT	2877	A-150787	AGUUCAGAAGAUUGGAUAAdTdT	3157	UUAUCCAAUCUUCUGAACU	3437
AD-75141	A-150788	AAGAGCAUGGAGAAUAAACdTdT	2878	A-150789	GUUUAUUCUCCAUGCUCUUdTdT	3158	AAGAGCAUGGAGAAUAAAC	3438
AD-75142	A-150790	ACGCGGGAAAAAAGAUCUUdTdT	2879	A-150791	AAGAUCUUUUUUCCCGCGUdTdT	3159	ACGCGGGAAAAAAGAUCUU	3439
AD-75143	A-150792	GAUCUUAUAGGCAAAUAGAdTdT	2880	A-150793	UCUAUUUGCCUAUAAGAUCdTdT	3160	GAUCUUAUAGGCAAAUAGA	3440
AD-75144	A-150794	AAGAAUUUAAAAGAUAAGUdTdT	2881	A-150795	ACUUAUCUUUUAAAUUCUUdTdT	3161	AAGAAUUUAAAAGAUAAGU	3441
AD-75145	A-150796	GUAAGUUCCUUAUUGAUUUdTdT	2882	A-150797	AAAUCAAUAAGGAACUUACdTdT	3162	GUAAGUUCCUUAUUGAUUU	3442
AD-75146	A-150798	UUUUGUGCACUCUGCUCUAdTdT	2883	A-150799	UAGAGCAGAGUGCACAAAAdTdT	3163	UUUUGUGCACUCUGCUCUA	3443
AD-75147	A-150800	AAACAGAUAUUCAGCAAGUdTdT	2884	A-150801	ACUUGCUGAAUAUCUGUUUdTdT	3164	AAACAGAUAUUCAGCAAGU	3444
AD-75148	A-150802	UCAGCAAGUGGAGAAAAUAdTdT	2885	A-150803	UAUUUUCUCCACUUGCUGAdTdT	3165	UCAGCAAGUGGAGAAAAUA	3445
AD-75149	A-150804	AAGAACAAAGAGAAAAAAUdTdT	2886	A-150805	AUUUUUUCUCUUUGUUCUUdTdT	3166	AAGAACAAAGAGAAAAAAU	3446

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-75150	A-150806	AUACAUAGAUUUACCUGCAdTdT	2887	A-150807	UGCAGGUAAAUCUAUGUAUdTdT	3167	AUACAUAGAUUUACCUGCA	3447
AD-75151	A-150808	UUACCUGCAAAAAAUAGCUdTdT	2888	A-150809	AGCUAUUUUUUGCAGGUAAdTdT	3168	UUACCUGCAAAAAAUAGCU	3448
AD-75152	A-150810	UUUAUAGAAGACAUUCUCAdTdT	2889	A-150811	UGAGAAUGUCUUCUAUAAAdTdT	3169	UUUAUAGAAGACAUUCUCC	3449
AD-75153	A-150812	AGACAUCUCAAAGAGCAGUdTdT	2890	A-150813	ACUGCUCUUUGAGAUGUCUdTdT	3170	AGACAUCUCAAAGAGCAGU	3450
AD-75154	A-150814	UAUGAGAUGGGGGUUAUCUdTdT	2891	A-150815	AGAUAACCCCCAUCUCAUAdTdT	3171	UAUGAGAUGGGGGUUAUCU	3451
AD-75155	A-150816	CUACUGAUAAAGAAAGAAUdTdT	2892	A-150817	AUUCUUUCUUUAUCAGUAGdTdT	3172	CUACUGAUAAAGAAAGAAU	3452
AD-75156	A-150818	AAAGAAUUUAUGAGAAAUUdTdT	2893	A-150819	AAUUUCUCAUAAAAUCUUUdTdT	3173	AAAGAAUUUAUGAGAAAUU	3453
AD-75157	A-150820	UAACAAUCUGUGAAGAUUUdTdT	2894	A-150821	AAAUCUUCACAGAUUGUUAdTdT	3174	UAACAAUCUGUGAAGAUUU	3454
AD-75158	A-150822	UUUUACUUUAUACAGUCUUdTdT	2895	A-150823	AAGACUGUAUAAAGUAAAAdTdT	3175	UUUUACUUUAUACAGUCUU	3455
AD-75159	A-150824	UUUAUGAAUUUCUUAAUGUdTdT	2896	A-150825	ACAUUAAGAAAUUCAUAAAdTdT	3176	UUUAUGAAUUUCUUAAUGU	3456
AD-75160	A-150826	UUAAUGUUCAAAAUGACUUdTdT	2897	A-150827	AAGUCAUUUUGAACAUUAAdTdT	3177	UUAAUGUUCAAAAUGACUU	3457
AD-75161	A-150828	UUCUUCUUUUUUUAUAUCAdTdT	2898	A-150829	UGAUAUAAAAAAAGAAGAAdTdT	3178	UUCUUCUUUUUUUAUAUCA	3458
AD-75162	A-150830	AGAAUGAGGAAUAAUAAGUdTdT	2899	A-150831	ACUUAUUAUUCCUCAUUCUdTdT	3179	AGAAUGAGGAAUAAUAAGU	3459
AD-75163	A-150832	UUAAACCCACAUAGACUCUdTdT	2900	A-150833	AGAGUCUAUGUGGGUUUAAdTdT	3180	UUAAACCCACAUAGACUCU	3460
AD-75164	A-150834	CUUUAAAACUAUAGGCUAAdTdT	2901	A-150835	UUAGCCUAUAGUUUUAAAGdTdT	3181	CUUUAAAACUAUAGGCUAG	3461
AD-75165	A-150836	AGAUAGAAAUGUAUGUUUAdTdT	2902	A-150837	UAAACAUACAUUUCUAUCUdTdT	3182	AGAUAGAAAUGUAUGUUUG	3462
AD-75166	A-150838	UUUGACUUGUUGAAGCUAUdTdT	2903	A-150839	AUAGCUUCAACAAGUCAAAdTdT	3183	UUUGACUUGUUGAAGCUAU	3463
AD-75167	A-150840	UUUUUAAUCUUAAAAAGAUUdTdT	2904	A-150841	AAUCUUUUAAGAUUAAAAAdTdT	3184	UUUUUAAUCUUAAAAAGAUU	3464
AD-75168	A-150842	UUGUGCUAAUUUAUUAGAAdTdT	2905	A-150843	UUCUAAUAAAUUAGCACACAAdTdT	3185	UUGUGCUAAUUUAUUAGAG	3465
AD-75169	A-150844	UUAUUAGAGCAGAACCUGUdTdT	2906	A-150845	ACAGGUUCUGCUCUAAUAAdTdT	3186	UUAUUAGAGCAGAACCUGU	3466
AD-75170	A-150846	GUUUGGCUCUCCUCAGAAAdTdT	2907	A-150847	UUUCUGAGGAGAGCCAAACdTdT	3187	GUUUGGCUCUCCUCAGAAG	3467
AD-75171	A-150848	CAAUAUUUUCAAAAGAUAAdTdT	2908	A-150849	UUAUCUUUUGAAAAUAUUGdTdT	3188	CAAUAUUUUCAAAAGAUAA	3468
AD-75172	A-150850	UCAAAAGAUAAAAUCUGAUUdTdT	2909	A-150851	AAUCAGAUUUAUUCUUUUGAdTdT	3189	UCAAAAGAUAAAAUCUGAUU	3469
AD-75173	A-150852	UUAUGCAAUGGCAUCAUUUdTdT	2910	A-150853	AAAUGAUGCCAUUGCAUAAdTdT	3190	UUAUGCAAUGGCAUCAUUU	3470
AD-75174	A-150854	UGCAAUGGCAUCAUUUAUUdTdT	2911	A-150855	AAUAAAUGAUGCCAUUGCAdTdT	3191	UGCAAUGGCAUCAUUUAUU	3471
AD-75175	A-150856	UUUAAAACAGAAGAAAUUGUdTdT	2912	A-150857	ACAAUUCUUCUGUUUUAAAdTdT	3192	UUUAAAACAGAAGAAAUUGU	3472
AD-75176	A-150858	AACAACAAAAGGAAAAUGUdTdT	2913	A-150859	ACAUUUUCCUUUUGUUGUUdTdT	3193	AACAACAAAAGGAAAAUGU	3473

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-75177	A-150860	UUAAUCCUGUAGUACAUAUdTdT	2914	A-150861	AUAUGUACUACAGGAUUAAdTdT	3194	UUAAUCCUGUAGUACAUAU	3474
AD-75178	A-150862	UUUAAUAUUUUAUAAGACAdTdT	2915	A-150863	UGUCUUAUAAAAUAUUAAAdTdT	3195	UUUAAUAUUUUAUAAGACC	3475
AD-75179	A-150864	CCUUCCUGUUAGGUAUUAAdTdT	2916	A-150865	UUAAUACCUAACAGGAAGGdTdT	3196	CCUUCCUGUUAGGUAUUAG	3476
AD-75180	A-150866	UUAGGUAUUAGAAAGUGAUdTdT	2917	A-150867	AUCACUUUCUAAUACCUAAdTdT	3197	UUAGGUAUUAGAAAGUGAU	3477
AD-75181	A-150868	AUACAUAGAUAUCUUUUUUUdTdT	2918	A-150869	AAAAAAGAUAUCUAUGUAUdTdT	3198	AUACAUAGAUAUCUUUUUU	3478
AD-75182	A-150870	UUUUUGUGUAAUUUCUAUUdTdT	2919	A-150871	AAUAGAAAUUACACAAAAAdTdT	3199	UUUUUGUGUAAUUUCUAUU	3479
AD-75183	A-150872	UUAAAAAAGAGAGAAGACUdTdT	2920	A-150873	AGUCUUCUCUCUUUUUUAAdTdT	3200	UUAAAAAAGAGAGAAGACU	3480
AD-75184	A-150874	CUGUCAGAAGCUUUAAGUAdTdT	2921	A-150875	UACUUAAAGCUUCUGACAGdTdT	3201	CUGUCAGAAGCUUUAAGUG	3481
AD-75185	A-150876	UAUGGUACAGGAUAAAGAUdTdT	2922	A-150877	AUCUUUAUCCUGUACCAUAdTdT	3202	UAUGGUACAGGAUAAAGAU	3482
AD-75186	A-150878	UUAAAUAACCAAUUCCUAUdTdT	2923	A-150879	AUAGGAAUUGGUUAUUUAAdTdT	3203	UUAAAUAACCAAUUCCUAU	3483
AD-75187	A-150880	UUGUUUUUUAAAGAAACCUdTdT	2924	A-150881	AGGUUUCUUUAAAAAACAAdTdT	3204	UUGUUUUUUAAAGAAACCU	3484
AD-75188	A-150882	CUCUCACAGAUAAGACAGAdTdT	2925	A-150883	UCUGUCUUAUCUGUGAGAGdTdT	3205	CUCUCACAGAUAAGACAGA	3485
AD-75189	A-150884	CAGAAUUUUAUAGAGGGCUdTdT	2926	A-150885	AGCCCUCUAUAAAAUUCUGdTdT	3206	CAGAAUUUUAUAGAGGGCU	3486
AD-75190	A-150886	UCUAGAAUUAAAGGAACCUdTdT	2927	A-150887	AGGUUCCUUUAAUUCUAGAdTdT	3207	UCUAGAAUUAAAGGAACCU	3487
AD-75191	A-150888	CUCACUGAAAACAUAUAUUdTdT	2928	A-150889	AAUAUAUGUUUUCAGUGAGdTdT	3208	CUCACUGAAAACAUAUAUU	3488
AD-75192	A-150890	AAACAUAUAUUUCACGUGUdTdT	2929	A-150891	ACACGUGAAAUAUAUGUUUdTdT	3209	AAACAUAUAUUUCACGUGU	3489
AD-75193	A-150892	GUUCCCUCUUUUUUUUUUUdTdT	2930	A-150893	AAAAAAAAAAAAGAGGGAACdTdT	3210	GUUCCCUCUUUUUUUUUUU	3490
AD-75194	A-150894	UUAAGCGAUUCUCCCUGCCUdTdT	2931	A-150895	AGGCAGGGAGAAUCGCUUAAdTdT	3211	UUAAGCGAUUCUCCCUGCCU	3491
AD-75195	A-150896	CGGCUAAUUUUUUGGAUUUdTdT	2932	A-150897	AAAUCCAAAAAAAUUAGCCGdTdT	3212	CGGCUAAUUUUUUGGAUUU	3492
AD-75196	A-150898	UUUAAUAGAGACGGGGUUUdTdT	2933	A-150899	AAACCCCGUCUCUAUUAAAdTdT	3213	UUUAAUAGAGACGGGGUUU	3493
AD-75197	A-150900	UUUACCAUGUUGGCCAGGUdTdT	2934	A-150901	ACCUGGCCAACAUGGUAAAdTdT	3214	UUUACCAUGUUGGCCAGGU	3494
AD-75198	A-150902	UUGCUGGGAUUACAGGCAUdTdT	2935	A-150903	AUGCCUGUAAUCCCAGCAAdTdT	3215	UUGCUGGGAUUACAGGCAU	3495
AD-75199	A-150904	UUAAACAUGAUCCUUCUCUdTdT	2936	A-150905	AGAGAAGGAUCAUGUUUAAdTdT	3216	UUAAACAUGAUCCUUCUCU	3496
AD-75200	A-150906	GGGGUCUUUCAAGGGAAAdTdT	2937	A-150907	UUUCCCCCUUGAAAGACCCCdTdT	3217	GGGGUCUUUCAAGGGAAA	3497
AD-75201	A-150908	AAAAAUCCAAGCUUUUUUAdTdT	2938	A-150909	UAAAAAAGCUUGGAUUUUUdTdT	3218	AAAAAUCCAAGCUUUUUUA	3498
AD-75202	A-150910	AAAGUAAAAAAAAAAAAAAGdTdT	2939	A-150911	CUUUUUUUUUUUUUACUUUdTdT	3219	AAAGUAAAAAAAAAAAAAG	3499
AD-75203	A-150912	AGAGAGGACACAAAACCAAdTdT	2940	A-150913	UUGGUUUUGUGUCCUCUCUdTdT	3220	AGAGAGGACACAAAACCAA	3500

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-75204	A-150914	UUAAGAUGGAGACAGAGUUdTdT	2941	A-150915	AACUCUGUCUCCAUCUUAAdTdT	3221	UUAAGAUGGAGACAGAGUU	3501
AD-75205	A-150916	UUUCUCCUAAUAACCGGAAdTdT	2942	A-150917	UUCCGGUUAUUAGGAGAAAdTdT	3222	UUUCUCCUAAUAACCGGAG	3502
AD-75206	A-150918	GCUGAAUUACCUUUCACUUdTdT	2943	A-150919	AAGUGAAAGGUAAUUCAGCdTdT	3223	GCUGAAUUACCUUUCACUU	3503
AD-75207	A-150920	UUCAAAAACAUGACCUUCAdTdT	2944	A-150921	UGAAGGUCAUGUUUUUGAAdTdT	3224	UUCAAAAACAUGACCUUCC	3504
AD-75208	A-150922	CAAUCCUUAGAAUCUGCCUdTdT	2945	A-150923	AGGCAGAUUCUAAGGAUUGdTdT	3225	CAAUCCUUAGAAUCUGCCU	3505
AD-75209	A-150924	UUUUAUAUUACUGAGGCCUdTdT	2946	A-150925	AGGCCUCAGUAAUAUAAAAdTdT	3226	UUUUAUAUUACUGAGGCCU	3506
AD-75210	A-150926	AAAAGUAAACAUUACUCAUdTdT	2947	A-150927	AUGAGUAAUGUUUACUUUUdTdT	3227	AAAAGUAAACAUUACUCAU	3507
AD-75211	A-150928	UUUAUUUUGCCCAAAAUGAdTdT	2948	A-150929	UCAUUUUGGGCAAAAUAAAAdTdT	3228	UUUAUUUUGCCCAAAAUGC	3508
AD-75212	A-150930	CACUGAUGUAAAGUAGGAAdTdT	2949	A-150931	UUCCUACUUUACACUCAGUGdTdT	3229	CACUGAUGUAAAGUAGGAA	3509
AD-75213	A-150932	AAAAUAAAAACAGAGCUCUdTdT	2950	A-150933	AGAGCUCUGUUUUUAUUUUdTdT	3230	AAAAUAAAAACAGAGCUCU	3510
AD-75214	A-150934	CUAAAAUCCCUUUCAAGCAdTdT	2951	A-150935	UGCUUGAAAGGGAUUUUAGdTdT	3231	CUAAAAUCCCUUUCAAGCC	3511
AD-75215	A-150936	UUGACCCCACUCACCAACUdTdT	2952	A-150937	AGUUGGUGAGUGGGGUCAAdTdT	3232	UUGACCCCACUCACCAACU	3512
AD-75216	A-150938	UUAUCUUGUACCCGCUGCUdTdT	2953	A-150939	AGCAGCGGUACAAGAUAAdTdT	3233	UUAUCUUGUACCCGCUGCU	3513
AD-75217	A-150940	CUGAAACCUCAAGCUGUCUdTdT	2954	A-150941	AGACAGCUUGAGGUUUUCAGdTdT	3234	CUGAAACCUCAAGCUGUCU	3514
AD-75218	A-150942	GUAUCAUGAAAAUGUCUAUdTdT	2955	A-150943	AUAGACAUUUUUCAUGAUACdTdT	3235	GUAUCAUGAAAAUGUCUAU	3515
AD-75219	A-150944	UUCAAAAUAUCAAAACCUUdTdT	2956	A-150945	AAGGUUUUGAUAUUUUGAAdTdT	3236	UUCAAAAUAUCAAAACCUU	3516
AD-75220	A-150946	UUUCAAAUAUCACGCAGCUdTdT	2957	A-150947	AGCUGCGUGAUAUUUGAAAdTdT	3237	UUUCAAAUAUCACGCAGCU	3517
AD-75221	A-150948	CUUAUAUUCAGUUUACAUAdTdT	2958	A-150949	UAUGUAAACUGAAUAUAAGdTdT	3238	CUUAUAUUCAGUUUACAUA	3518
AD-75222	A-150950	UUACAUAAAGGCCCCAAAUdTdT	2959	A-150951	AUUUGGGGCCUUUAUGUAAdTdT	3239	UUACAUAAAGGCCCCAAAU	3519
AD-75223	A-150952	AUACCAUGUCAGAUCUUUUdTdT	2960	A-150953	AAAAGAUCUGACAUGGUAUdTdT	3240	AUACCAUGUCAGAUCUUUU	3520
AD-75224	A-150954	AAAAGAGUUAAUGAACUAUdTdT	2961	A-150955	AUAGUUCAUUAACUCUUUUdTdT	3241	AAAAGAGUUAAUGAACUAU	3521
AD-75225	A-150956	AUGAGAAUUGGGAUUACAUdTdT	2962	A-150957	AUGUAAUCCCAAUUCUCAUdTdT	3242	AUGAGAAUUGGGAUUACAU	3522
AD-75226	A-150958	AUCAUGUAUUUUGCCUCAUdTdT	2963	A-150959	AUGAGGCAAAAUACAUGAUdTdT	3243	AUCAUGUAUUUUGCCUCAU	3523
AD-75227	A-150960	UUAUCACACUUAUAGGCCAdTdT	2964	A-150961	UGGCCUAUAAGUGUGAUAAdTdT	3244	UUAUCACACUUAUAGGCCA	3524
AD-75228	A-150962	CAAGUGUGAUAAAUAAACUdTdT	2965	A-150963	AGUUUAUUUAUCACACUUGdTdT	3245	CAAGUGUGAUAAAUAAACU	3525
AD-75229	A-150964	UUACAGACACUGAAUUAAUdTdT	2966	A-150965	AUUAAUUCAGUGUCUGUAAdTdT	3246	UUACAGACACUGAAUUAAU	3526
AD-75230	A-150966	UUUGAAACCAGAAAAAUAAUdTdT	2967	A-150967	AUUAUUUUCUGGUUUCAAAdTdT	3247	UUUGAAACCAGAAAAAUAAU	3527

Duplex Name	Sense Oligo Name	Sense Sequence	SEQ ID NO	Antisense Oligo Name	Antisense Sequence	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-75231	A-150968	AUGACUGGCCAUUCGUUAAdTdT	2968	A-150969	UUAACGAAUGGGCCAGUCAUdTdT	3248	AUGACUGGCCAUUCGUUAC	3528
AD-75232	A-150970	UUAGUUGAAAAGCAUAUUUdTdT	2969	A-150971	AAAUAUGCUUUUCAACUAAdTdT	3249	UUAGUUGAAAAGCAUAUUU	3529
AD-75233	A-150972	UUUUUAUUAAAUUAAUUCUdTdT	2970	A-150973	AGAAUUAAUUUAAUAAAAAdTdT	3250	UUUUUAUUAAAUUAAUUCU	3530
AD-75234	A-150974	CUGAUUGUAUUUGAAAUUAdTdT	2971	A-150975	UAAUUUCAAAUACAAUCAGdTdT	3251	CUGAUUGUAUUUGAAAUUA	3531
AD-75235	A-150976	UUUGAAAUUAUUAUUCAAUdTdT	2972	A-150977	AUUGAAUAAUAAUUUCAAAdTdT	3252	UUUGAAAUUAUUAUUCAAU	3532
AD-75236	A-150978	UUAUGGCAGAGGAAUAUCAdTdT	2973	A-150979	UGAUAUUCCUCUGCCAUAAdTdT	3253	UUAUGGCAGAGGAAUAUCA	3533
AD-75237	A-150980	UCUAAAAAAUGUAACUAAUUdTdT	2974	A-150981	AAUUAGUUACAUUUUUUAGAdTdT	3254	UCUAAAAAAUGUAACUAAUU	3534
AD-75238	A-150982	UUUACUGUUUAAUAAGCAUdTdT	2975	A-150983	AUGCUUAUUAAACAGUAAAdTdT	3255	UUUACUGUUUAAUAAGCAU	3535
AD-75239	A-150984	UGUCAUAAUAAAAAUGGUAUdTdT	2976	A-150985	AUACCAUUUUAUUAUGACAdTdT	3256	UGUCAUAAUAAAAAUGGUAU	3536
AD-75240	A-150986	AUAUCUUUCUUUAGUAAUUdTdT	2977	A-150987	AAUUACUAAAGAAAGAUAUdTdT	3257	AUAUCUUUCUUUAGUAAUU	3537
AD-75241	A-150988	UUAGUAAUUACAUUAAAAAUdTdT	2978	A-150989	AUUUUAAUGUAAUUACUAAdTdT	3258	UUAGUAAUUACAUUAAAAAU	3538
AD-75242	A-150990	AUUAGUCAUGUUUGAUUAAdTdT	2979	A-150991	UUAAUCAAACAUGACUAAUdTdT	3259	AUUAGUCAUGUUUGAUUAA	3539

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of an insulin-like growth factor binding protein, acid labile subunit (IGFALS) gene, wherein the dsRNA agent comprises a sense strand and an antisense strand, wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of any one of SEQ ID NO:1, 3, or 5, wherein said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of any one of SEQ ID NO:2, 4, or 6; and wherein the dsRNA agent comprises at least one modified nucleotide.

2. The dsRNA agent of claim 1, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of nucleotides 2052-2084, 11-62, 24-62, 79-117, 79-130, 155-173, 194-216, 194-229, 211-229, 232-293, 254-272, 310-328, 310-349, 324-345, 331-349, 353-371, 353-394, 376-394, 407-425, 439-449, 431-470, 484-515, 497-515, 541-580, 547-568, 596-647, 616-634, 673-691, 694-712, 694-734, 777-797, 781-799, 825-843, 825-855, 869-922, 958-976, 958-988, 1064-1085, 1064-1096, 1067-1085, 1067-1096, 1100-1141, 1111-1129, 1145-1163, 1145-1186, 1159-1186, 1168-1196, 1168-1214,1193-1214, 1266-1307, 1321-1339, 1342-1373, 1375-1406, 1432-1450, 1454-1472, 1519-1537, 1519-1559, 1534-1555, 1541-1559, 1606-1624, 1606-1637, 1613-1635, 1672-1690, 1672-1712, 1749-1779, 1783-1801, 1805-1823, 1806-1829, 1871-1889, 1871-1919, 1949-1977, 1993-2011, 2013-2042, 2048-2077, or 2048-2088 of the nucleotide sequence of SEQ ID NO:1.

3. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of an insulin-like growth factor binding protein, acid labile subunit (IGFALS) gene, wherein said dsRNA agent comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementarity to a target IGFALS gene nucleotide sequence and comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense sequences listed in any one of Table 3, 5, 6, 8, 12, or 14.

4. The dsRNA agent of claim 3, wherein the nucleotide sequence of the antisense strand is selected from the group consisting of the antisense nucleotide sequences of any one of duplexes AD-68730, AD-62728, AD-62734, AD-68111, AD-68709, AD-68712, AD-68715, AD-68716, AD-68717, AD-68719, AD-68720, AD-68722, AD-68725, AD-68726, AD-68731, AD-73782, AD-73773, AD-73765, AD-73946, AD-73947, AD-73858, AD-73797, AD-73808, AD-73906, AD-73912, AD-73848, AD-73836, AD-73818, AD-73786, AD-73862, AD-73795, AD-73766, AD-73930, AD-73825, AD-73924, AD-73802, AD-73767, AD-73771, AD-73777, AD-73793, AD-73898, AD-73784, AD-73882, AD-73803, AD-73772, AD-73907, AD-73948, AD-73890, AD-73883, AD-73770, AD-73867, AD-73931, AD-73932, AD-73787, AD-73791, AD-73880, AD-73914, AD-73849, AD-73863, AD-73920, AD-73944, AD-73841, AD-73785, AD-73804, AD-73823, AD-73885, AD-73788, AD-73865, AD-

73941, AD-73859, AD-73913, AD-73892, AD-73837, AD-73842, AD-73840, AD-73813, AD-73796, AD-73875, AD-73900, AD-73922, AD-73861, AD-73816, AD-73764, AD-73868, AD-73812, AD-73826, AD-73938, AD-73843, AD-73817, AD-73943, AD-73827, AD-73937, AD-73877, AD-73833, AD-73807, AD-73819, AD-73886, AD-73919, AD-73800, AD-76171, AD-76173, AD-76203, AD-76210, AD-76172, AD-76175, AD-76209, AD-76174, AD-76208, AD-76186, AD-76177, AD-76199, AD-76197, and AD-76212.

5. The dsRNA agent of claim 1, wherein the sense and antisense strands comprise nucleotide sequences selected from the group consisting of any one of the nucleotide sequences in any one of Tables 3, 5, 6, 8, 12, or 14.

6. The dsRNA agent of claim 5, wherein the sense and antisense nucleotide sequences are selected from the group consisting of the sense and antisense nucleotide sequences of any one of duplexes AD-62728, AD-62734, AD-68111, AD-68709, AD-68712, AD-68715, AD-68716, AD-68717, AD-68719, AD-68720, AD-68722, AD-68725, AD-68726, AD-68730, AD-68731, AD-73782, AD-73773, AD-73765, AD-73946, AD-73947, AD-73858, AD-73797, AD-73808, AD-73906, AD-73912, AD-73848, AD-73836, AD-73818, AD-73786, AD-73862, AD-73795, AD-73766, AD-73930, AD-73825, AD-73924, AD-73802, AD-73767, AD-73771, AD-73777, AD-73793, AD-73898, AD-73784, AD-73882, AD-73803, AD-73772, AD-73907, AD-73948, AD-73890, AD-73883, AD-73770, AD-73867, AD-73931, AD-73932, AD-73787, AD-73791, AD-73880, AD-73914, AD-73849, AD-73863, AD-73920, AD-73944, AD-73841, AD-73785, AD-73804, AD-73823, AD-73885, AD-73788, AD-73865, AD-73941, AD-73859, AD-73913, AD-73892, AD-73837, AD-73842, AD-73840, AD-73813, AD-73796, AD-73875, AD-73900, AD-73922, AD-73861, AD-73816, AD-73764, AD-73868, AD-73812, AD-73826, AD-73938, AD-73843, AD-73817, AD-73943, AD-73827, AD-73937, AD-73877, AD-73833, AD-73807, AD-73819, AD-73886, AD-73919, AD-73800, AD-76171, AD-76173, AD-76203, AD-76210, AD-76172, AD-76175, AD-76209, AD-76174, AD-76208, AD-76186, AD-76177, AD-76199, AD-76197, and AD-76212.

7. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of an insulin-like growth factor 1 (IGF-1) gene, wherein said dsRNA agent comprises a sense strand and an antisense strand, wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of any one of SEQ ID NO:11 or 13, wherein said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of any one of SEQ ID NO: 12 or 14; and wherein the dsRNA agent comprises at least one modified nucleotide.

8. The dsRNA agent of claim 7, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of nucleotides 328-369, 340-369, 430-490, 430-482, 434-460, 532-559, 330-350, 342-362, 348-368, 349-369, 432-452, 435-455, 436-

456, 438-458, 440-460, 441-461, 442-462, 449-469, 455-475, 460-480, 461-481, 462-482, 464-484, 470-490, 484-501, 534-554, 536-556, 538-558, 539-559, 542-562, 548-568, 577-597, 582-602, or 640-660 of the nucleotide sequence of SEQ ID NO:11; or wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of nucleotides 6-90, 127-145, 185-238, 247-265, 277-295, 389-417, 430-480, 543-561, 654-690, 750-768, 774-870, 894-930, 1007-1029, 1075-1126, 1144-1162, 1197-1215, 1232-1250, 1293-1311, 1334-1352, 1388-1458, 1463-1490, 1511-1529, 1599-1617, 1643-1661, 1690-1727, 1793-1825, 1843-1861, 2057-2075, 2090-2130, 2192-2228, 2310-2332, 2357-2375, 2521-2539, 2566-2588, 2648-2684, 2793-2811, 2962-2980, 3120-3142, 3208-3233, 3269-3287, 3417-3435, 3449-3467, 3575-3603, 3686-3704, 3721-3739, 3806-3824, 3939-3957, 3982-4018, 4081-4037, 4154-4172, 4271-4289, 4319-4377, 4436-4478, 4484-4502, 4523-4545, 4566-4584, 4610-4660, 4686-4717, 4734-4769, 4780-4798, 4815-4843, 4884-4902, 4911-4929, 5004-5034, 5050-5068, 5171-5256, 5311-5364, 5409-5430, 5551-5588, 5609-5638, 5694-5712, 5715-5758, 5790-5808, 5906-5928, 5934-5952, 6323-6345, 6399-6417, 6461-6497, 6510-6535, 6584-6612, 6629-6647, 6661-6683, 6726-6789, 6796-6824, 6826-6851, 6858-6905, 6910-6927, 7004-7022, 7035-7130, 7144-7162, 7175-7241, or 7252-7270 of the nucleotide sequence of SEQ ID NO:13.

9. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of an insulin-like growth factor 1 (IGF-1) gene, wherein said dsRNA agent comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementarity to a target IGF-1 gene nucleotide sequence and comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense sequences listed in any one of Tables 9, 11, 15, 17, 18, or 20.

10. The dsRNA agent of claim 9, wherein the nucleotide sequence of the antisense strand is selected from the group consisting of any one of the antisense nucleotide sequences of any one of duplexes AD-66722, AD-66748, AD-66746, AD-66747, AD-66733, AD-66752, AD-66739, AD-66738, AD-66725, AD-66740, AD-66750, AD-66729, AD-66745, AD-66749, AD-66720, AD-66724, AD-66726, AD-66766, AD-66761, AD-66755, AD-66751, AD-66719, AD-66727, AD-66744, AD-66760, AD-66753, AD-66721, AD-66716, AD-66743, AD-66728, AD-77150, AD-77158, AD-74963, AD-77138, AD-75740, AD-74968, AD-74965, AD-75766, AD-75761, AD-75137, AD-74979, AD-74966, AD-75750, AD-77126, AD-74971, AD-74982, AD-77144, AD-77149, AD-75751, AD-75111, AD-77147, AD-74964, AD-74983, AD-75765, AD-74970, AD-75749, AD-77168, AD-77127, AD-75748, AD-75779, AD-75145, AD-74975, AD-77151, AD-75170, AD-75741, AD-75162, AD-74985, AD-75759, AD-75218, AD-74981, AD-75155, AD-74978, AD-77153, AD-75157, AD-75123, AD-75184, AD-77160, AD-75125, AD-75229, AD-77165, AD-75112, AD-75206, AD-75769, AD-75174, AD-75225, AD-75792, AD-75115, AD-74986, AD-77171, AD-75131, AD-77128, AD-75179, AD-75792, AD-77124, AD-75191, AD-75774, AD-75114, AD-74973, AD-77156, AD-75120, AD-75130, AD-74967, AD-75231, AD-74987, AD-77140, AD-74969, AD-75000, AD-75791, AD-75143, AD-77120, AD-77142, AD-75217, AD-75234, AD-75173, AD-75232, AD-75188, AD-75135, AD-75018,

AD-77122, AD-75009, AD-75121, AD-75791, AD-77135, AD-75214, AD-74994, AD-75139, AD-75166, AD-75020, AD-77159, AD-75236, AD-77123, AD-77133, AD-74972, AD-75223, AD-75148, AD-75124, AD-75185, AD-75150, AD-74976, AD-74980, AD-75212, AD-75239, AD-75221, AD-75118, AD-75793, AD-75023, AD-75164, AD-74997, AD-74984, AD-75011, AD-75203, AD-77161, AD-75033, AD-75177, AD-75795, AD-77146, AD-75793, AD-75788, AD-75079, AD-75152, AD-77121, AD-75237, AD-75014, AD-75755, AD-75028, AD-75091, AD-75110, AD-75230, AD-75029, AD-75099, AD-77130, AD-75224, AD-75142, AD-75760, AD-75795, AD-77136, AD-75032, AD-75757, AD-75017, AD-75151, AD-75122, AD-75002, AD-75021, AD-75005, AD-75088, AD-75153, AD-75208, AD-74977, AD-75069, AD-75107, AD-74990, AD-75061, AD-75083, AD-75116, AD-75169, AD-75058, AD-74991, AD-75041, AD-77131, AD-75772, AD-77169, AD-75133, AD-75222, AD-75007, AD-75101, AD-77137, AD-75090, AD-77148, AD-75008, AD-77134, AD-74999, AD-75048, AD-75095, AD-74974, AD-75788, AD-75057, AD-75113, AD-77172, AD-75016, AD-75186, AD-75205, AD-75238, and AD-75146 .

11. The dsRNA agent of claim 7, wherein the sense and antisense strands comprise nucleotide sequences selected from the group consisting of any one of the nucleotide sequences in any one of Tables 9, 11, 15, 17, 18, or 20.

12. The dsRNA agent of claim 11, wherein the sense and antisense nucleotide sequences are selected from the group consisting of any one of the sense and antisense nucleotide sequences of any one of duplexes AD-66722, AD-66748, AD-66746, AD-66747, AD-66733, AD-66752, AD-66739, AD-66738, AD-66725, AD-66740, AD-66750, AD-66729, AD-66745, AD-66749, AD-66720, AD-66724, AD-66726, AD-66766, AD-66761, AD-66755, AD-66751, AD-66719, AD-66727, AD-66744, AD-66760, AD-66753, AD-66721, AD-66716, AD-66743, AD-66728, AD-77150, AD-77158, AD-74963, AD-77138, AD-75740, AD-74968, AD-74965, AD-75766, AD-75761, AD-75137, AD-74979, AD-74966, AD-75750, AD-77126, AD-74971, AD-74982, AD-77144, AD-77149, AD-75751, AD-75111, AD-77147, AD-74964, AD-74983, AD-75765, AD-74970, AD-75749, AD-77168, AD-77127, AD-75748, AD-75779, AD-75145, AD-74975, AD-77151, AD-75170, AD-75741, AD-75162, AD-74985, AD-75759, AD-75218, AD-74981, AD-75155, AD-74978, AD-77153, AD-75157, AD-75123, AD-75184, AD-77160, AD-75125, AD-75229, AD-77165, AD-75112, AD-75206, AD-75769, AD-75174, AD-75225, AD-75792, AD-75115, AD-74986, AD-77171, AD-75131, AD-77128, AD-75179, AD-75792, AD-77124, AD-75191, AD-75774, AD-75114, AD-74973, AD-77156, AD-75120, AD-75130, AD-74967, AD-75231, AD-74987, AD-77140, AD-74969, AD-75000, AD-75791, AD-75143, AD-77120, AD-77142, AD-75217, AD-75234, AD-75173, AD-75232, AD-75188, AD-75135, AD-75018, AD-77122, AD-75009, AD-75121, AD-75791, AD-77135, AD-75214, AD-74994, AD-75139, AD-75166, AD-75020, AD-77159, AD-75236, AD-77123, AD-77133, AD-74972, AD-75223, AD-75148, AD-75124, AD-75185, AD-75150, AD-74976, AD-74980, AD-75212, AD-75239, AD-75221, AD-75118, AD-75793, AD-75023, AD-75164, AD-74997, AD-74984, AD-75011, AD-75203, AD-77161, AD-75033, AD-75177, AD-75795, AD-77146, AD-75793, AD-75788, AD-75079, AD-75152,

AD-77121, AD-75237, AD-75014, AD-75755, AD-75028, AD-75091, AD-75110, AD-75230, AD-75029, AD-75099, AD-77130, AD-75224, AD-75142, AD-75760, AD-75795, AD-77136, AD-75032, AD-75757, AD-75017, AD-75151, AD-75122, AD-75002, AD-75021, AD-75005, AD-75088, AD-75153, AD-75208, AD-74977, AD-75069, AD-75107, AD-74990, AD-75061, AD-75083, AD-75116, AD-75169, AD-75058, AD-74991, AD-75041, AD-77131, AD-75772, AD-77169, AD-75133, AD-75222, AD-75007, AD-75101, AD-77137, AD-75090, AD-77148, AD-75008, AD-77134, AD-74999, AD-75048, AD-75095, AD-74974, AD-75788, AD-75057, AD-75113, AD-77172, AD-75016, AD-75186, AD-75205, AD-75238, and AD-75146.

13. The dsRNA agent of any one of claims 1-12, wherein substantially all of the nucleotides of said sense strand or substantially all of the nucleotides of said antisense strand comprise a nucleotide modification.

14. The dsRNA agent of any one of claims 1-12, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a nucleotide modification.

15. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of an insulin-like growth factor binding protein, acid labile subunit (IGFALS) gene, wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein said 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 said 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 said sense strand and substantially all of the nucleotides of said antisense strand comprise a nucleotide modification, and

wherein said sense strand is conjugated to a ligand attached at the 3'-terminus.

16. The dsRNA agent of claim 15, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of nucleotides 11-62, 24-62, 79-117, 79-130, 155-173, 194-216, 194-229, 211-229, 232-293, 254-272, 310-328, 310-349, 324-345, 331-349, 353-371, 353-394, 376-394, 407-425, 439-449, 431-470, 484-515, 497-515, 541-580, 547-568, 596-647, 616-634, 673-691, 694-712, 694-734, 777-797, 781-799, 825-843, 825-855, 869-922, 958-976, 958-988, 1064-1085, 1064-1096, 1067-1085, 1067-1096, 1100-1141, 1111-1129, 1145-1163, 1145-1186, 1159-1186, 1168-1196, 1168-1214,1193-1214, 1266-1307, 1321-1339, 1342-1373, 1375-1406, 1432-1450, 1454-1472, 1519-1537, 1519-1559, 1534-1555, 1541-1559, 1606-1624, 1606-1637, 1613-1635, 1672-1690, 1672-1712, 1749-1779, 1783-1801, 1805-1823, 1806-1829, 1871-1889, 1871-1919, 1949-1977, 1993-2011, 2013-2042, 2048-2077, 2048-2088, or 2052-2084 of the nucleotide sequence of SEQ ID NO:1.

17. The dsRNA agent of claim 15, wherein the sense and antisense nucleotide sequences are selected from the group consisting of any one of the sense and antisense nucleotide sequences of any one of duplexes AD-62728, AD-62734, AD-68111, AD-68709, AD-68712, AD-68715, AD-68716, AD-68717, AD-68719, AD-68720, AD-68722, AD-68725, AD-68726, AD-68730, AD-68731, AD-73782, AD-73773, AD-73765, AD-73946, AD-73947, AD-73858, AD-73797, AD-73808, AD-73906, AD-73912, AD-73848, AD-73836, AD-73818, AD-73786, AD-73862, AD-73795, AD-73766, AD-73930, AD-73825, AD-73924, AD-73802, AD-73767, AD-73771, AD-73777, AD-73793, AD-73898, AD-73784, AD-73882, AD-73803, AD-73772, AD-73907, AD-73948, AD-73890, AD-73883, AD-73770, AD-73867, AD-73931, AD-73932, AD-73787, AD-73791, AD-73880, AD-73914, AD-73849, AD-73863, AD-73920, AD-73944, AD-73841, AD-73785, AD-73804, AD-73823, AD-73885, AD-73788, AD-73865, AD-73941, AD-73859, AD-73913, AD-73892, AD-73837, AD-73842, AD-73840, AD-73813, AD-73796, AD-73875, AD-73900, AD-73922, AD-73861, AD-73816, AD-73764, AD-73868, AD-73812, AD-73826, AD-73938, AD-73843, AD-73817, AD-73943, AD-73827, AD-73937, AD-73877, AD-73833, AD-73807, AD-73819, AD-73886, AD-73919, AD-73800, AD-76171, AD-76173, AD-76203, AD-76210, AD-76172, AD-76175, AD-76209, AD-76174, AD-76208, AD-76186, AD-76177, AD-76199, AD-76197, and AD-76212.

18. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of an insulin-like growth factor-1 (IGF-1) gene, wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:11 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:12; or wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:13 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:14,

wherein substantially all of the nucleotides of said sense strand and substantially all of the nucleotides of said antisense strand comprise a nucleotide modification, and

wherein said sense strand is conjugated to a ligand attached at the 3'-terminus.

19. The dsRNA agent of claim 18, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of nucleotides 330-369, 342-369, 432-490, 432-482, 436-462, 534-559, 330-350, 342-362, 348-368, 349-369, 432-452, 435-455, 436-456, 438-458, 440-460, 441-461, 442-462, 449-469, 455-475, 460-480, 461-481, 462-482, 464-484, 470-490, 484-501, 534-554, 536-556, 538-558, 539-559, 542-562, 548-568, 577-597, 582-602, or 640-660 of the nucleotide sequence of SEQ ID NO:11; or wherein the wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of nucleotides 6-90, 127-145, 185-238, 247-265, 277-295, 389-417, 430-480, 543-561, 654-690, 750-

768, 774-870, 894-930, 1007-1029, 1075-1126, 1144-1162, 1197-1215, 1232-1250, 1293-1311, 1334-1352, 1388-1458, 1463-1490, 1511-1529, 1599-1617, 1643-1661, 1690-1727, 1793-1825, 1843-1861, 2057-2075, 2090-2130, 2192-2228, 2310-2332, 2357-2375, 2521-2539, 2566-2588, 2648-2684, 2793-2811, 2962-2980, 3120-3142, 3208-3233, 3269-3287, 3417-3435, 3449-3467, 3575-3603, 3686-3704, 3721-3739, 3806-3824, 3939-3957, 3982-4018, 4081-4037, 4154-4172, 4271-4289, 4319-4377, 4436-4478, 4484-4502, 4523-4545, 4566-4584, 4610-4660, 4686-4717, 4734-4769, 4780-4798, 4815-4843, 4884-4902, 4911-4929, 5004-5034, 5050-5068, 5171-5256, 5311-5364, 5409-5430, 5551-5588, 5609-5638, 5694-5712, 5715-5758, 5790-5808, 5906-5928, 5934-5952, 6323-6345, 6399-6417, 6461-6497, 6510-6535, 6584-6612, 6629-6647, 6661-6683, 6726-6789, 6796-6824, 6826-6851, 6858-6905, 6910-6927, 7004-7022, 7035-7130, 7144-7162, 7175-7241, or 7252-7270 of the nucleotide sequence of SEQ ID NO: 13.

20. The dsRNA agent of claim 18, wherein the sense and antisense nucleotide sequences are selected from the group consisting of any one of the sense and antisense nucleotide sequences of any one of duplexes AD-66722, AD-66748, AD-66746, AD-66747, AD-66733, AD-66752, AD-66739, AD-66738, AD-66725, AD-66740, AD-66750, AD-66729, AD-66745, AD-66749, AD-66720, AD-66724, AD-66726, AD-66766, AD-66761, AD-66755, AD-66751, AD-66719, AD-66727, AD-66744, AD-66760, AD-66753, AD-66721, AD-66716, AD-66743, and AD-66728; for example duplexes AD-66722, AD-66748, AD-66746, AD-66747, AD-66733, AD-66752, AD-66739, AD-66738, AD-66725, AD-66740, AD-66750, AD-66729, AD-66745, AD-77150, AD-77158, AD-74963, AD-77138, AD-75740, AD-74968, AD-74965, AD-75766, AD-75761, AD-75137, AD-74979, AD-74966, AD-75750, AD-77126, AD-74971, AD-74982, AD-77144, AD-77149, AD-75751, AD-75111, AD-77147, AD-74964, AD-74983, AD-75765, AD-74970, AD-75749, AD-77168, AD-77127, AD-75748, AD-75779, AD-75145, AD-74975, AD-77151, AD-75170, AD-75741, AD-75162, AD-74985, AD-75759, AD-75218, AD-74981, AD-75155, AD-74978, AD-77153, AD-75157, AD-75123, AD-75184, AD-77160, AD-75125, AD-75229, AD-77165, AD-75112, AD-75206, AD-75769, AD-75174, AD-75225, AD-75792, AD-75115, AD-74986, AD-77171, AD-75131, AD-77128, AD-75179, AD-75792, AD-77124, AD-75191, AD-75774, AD-75114, AD-74973, AD-77156, AD-75120, AD-75130, AD-74967, AD-75231, AD-74987, AD-77140, AD-74969, AD-75000, AD-75791, AD-75143, AD-77120, AD-77142, AD-75217, AD-75234, AD-75173, AD-75232, AD-75188, AD-75135, AD-75018, AD-77122, AD-75009, AD-75121, AD-75791, AD-77135, AD-75214, AD-74994, AD-75139, AD-75166, AD-75020, AD-77159, AD-75236, AD-77123, AD-77133, AD-74972, AD-75223, AD-75148, AD-75124, AD-75185, AD-75150, AD-74976, AD-74980, AD-75212, AD-75239, AD-75221, AD-75118, AD-75793, AD-75023, AD-75164, AD-74997, AD-74984, AD-75011, AD-75203, AD-77161, AD-75033, AD-75177, AD-75795, AD-77146, AD-75793, AD-75788, AD-75079, AD-75152, AD-77121, AD-75237, AD-75014, AD-75755, AD-75028, AD-75091, AD-75110, AD-75230, AD-75029, AD-75099, AD-77130, AD-75224, AD-75142, AD-75760, AD-75795, AD-77136, AD-75032, AD-75757, AD-75017, AD-75151, AD-75122, AD-75002, AD-75021, AD-75005, AD-75088, AD-75153, AD-75208, AD-74977, AD-75069, AD-75107, AD-74990, AD-75061, AD-75083, AD-75116, AD-75169, AD-75058,

AD-74991, AD-75041, AD-77131, AD-75772, AD-77169, AD-75133, AD-75222, AD-75007, AD-75101, AD-77137, AD-75090, AD-77148, AD-75008, AD-77134, AD-74999, AD-75048, AD-75095, AD-74974, AD-75788, AD-75057, AD-75113, AD-77172, AD-75016, AD-75186, AD-75205, AD-75238, and AD-75146.

21. The dsRNA agent of claim 15 or 18, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand are modified nucleotides.

22. The dsRNA agent of any of claim 1-21, wherein at least one of said modified nucleotides comprises a nucleotide modification 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'-hydroxly-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, and a nucleotide comprising a 5'-phosphate mimic.

23. The dsRNA agent of claim 22, wherein said modified nucleotide comprises a short sequence of 3'-terminal deoxy-thymine nucleotides (dT).

24. The dsRNA agent of claim 3 or 9, wherein the region of complementarity is at least 17 nucleotides in length.

25. The dsRNA agent of claim 3 or 9, wherein the region of complementarity is 19 -21 nucleotides in length.

26. The dsRNA agent of claim 25, wherein the region of complementarity is 19 nucleotides in length.

27. The dsRNA agent of any one of claims 1, 3, 7, 9, 15, and 18, wherein each strand is no more than 30 nucleotides in length.

28. The dsRNA agent of any one of claims 1, 3, 7, 9, 15, and 18, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

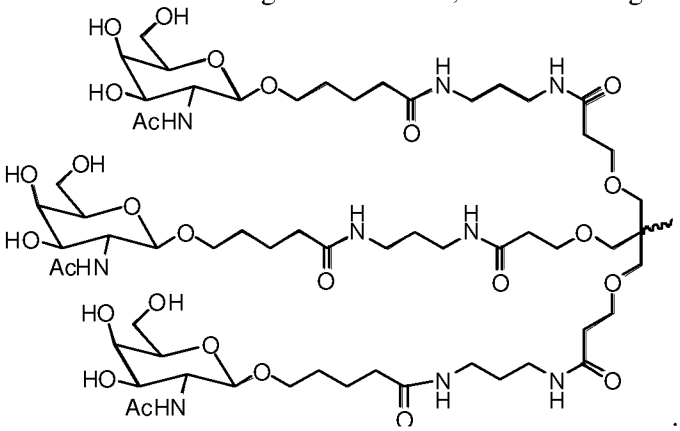
29. The dsRNA agent of any one of claims 1, 3, 7, 9, 15, and 18, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

30. The dsRNA agent of any one of claims 1-14 further comprising a ligand.

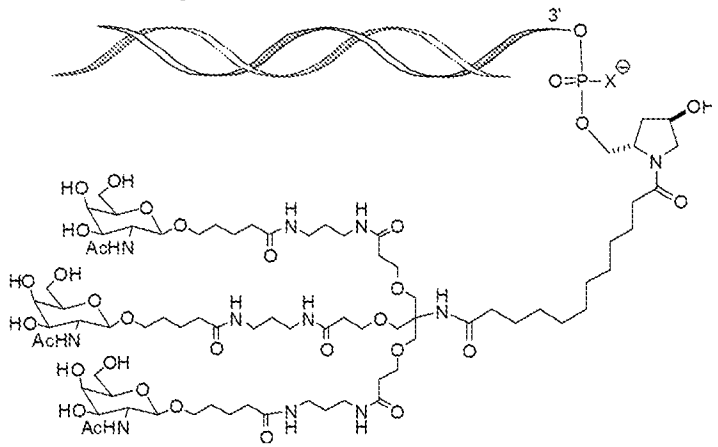
31. The dsRNA agent of claim 30, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

32. The dsRNA agent of claim 15, 18, or 30, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

33. The dsRNA agent of claim 32, wherein the ligand is



34. The dsRNA agent of claim 32, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

35. The dsRNA agent of claim 34, wherein the X is O.

36. The dsRNA agent of claim 3, wherein the region of complementarity comprises any one of the antisense nucleotide sequences listed in any one of Tables 3, 5, 6, 8, 12, or 14 .

37. The dsRNA agent of claim 3, wherein the region of complementarity consists of any one of the antisense nucleotide sequences listed in any one of Tables 3, 5, 6, 8, 12, or 14.

38. The dsRNA agent of claim 9, wherein the region of complementarity comprises any one of the antisense nucleotide sequences listed in any one of Tables 9, 11, 15, 17, 18, or 20.

39. The dsRNA agent of claim 9, wherein the region of complementarity consists of any one of the antisense nucleotide sequences listed in any one of Tables 9, 11, 15, 17, 18, or 20.

40. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of an insulin-like growth factor binding protein, acid labile subunit (IGFALS) gene or an insulin-like growth factor 1 (IGF-1) gene, wherein said dsRNA agent comprises a sense strand complementary to an antisense strand, wherein said antisense strand comprises a region of complementary to part of an mRNA encoding IGFALS or IGF-1, respectively, wherein each strand is about 14 to about 30 nucleotides in length, wherein said dsRNA agent is represented by formula (III):

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' (III)

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.

41. The dsRNA agent of claim 40, 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.

42. The dsRNA agent of claim 40, 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.

43. The dsRNA agent of claim 40, wherein XXX is complementary to X′X′X′, YYY is complementary to Y′Y′Y′, and ZZZ is complementary to Z′Z′Z′.

44. The dsRNA agent of claim 40, wherein the YYY motif occurs at or near the cleavage site of the sense strand.

45. The dsRNA agent of claim 40, wherein the Y′Y′Y′ motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end.

46. The dsRNA agent of claim 45, wherein the Y′ is 2′-O-methyl.

47. The dsRNA agent of claim 40, wherein formula (III) is represented by formula (IIIa):

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' (IIIa).

48. The dsRNA agent of claim 40, wherein formula (III) is represented by formula (IIIb):

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' (IIIb)

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

49. The dsRNA agent of claim 40, wherein formula (III) is represented by formula (IIIc):

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' (IIIc)

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

50. The dsRNA agent of claim 40, wherein formula (III) is represented by formula (IIId):

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' (IIId)

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.

51. The dsRNA agent of any one of claims 1-50, wherein the double stranded region is 15-30 nucleotide pairs in length.

52. The dsRNA agent of claim 51, wherein the double stranded region is 17-23 nucleotide pairs in length.

53. The dsRNA agent of claim 51, wherein the double stranded region is 17-25 nucleotide pairs in length.

54. The dsRNA agent of claim 51, wherein the double stranded region is 23-27 nucleotide pairs in length.

55. The dsRNA agent of claim 51, wherein the double stranded region is 19-21 nucleotide pairs in length.

56. The dsRNA agent of any one of claims 1-50, wherein the double stranded region is 21-23 nucleotide pairs in length.

57. The dsRNA agent of any one of claims 1-56, wherein each strand has 15-30 nucleotides.

58. The dsRNA agent of any one of claims 1-56, wherein each strand has 19-30 nucleotides.

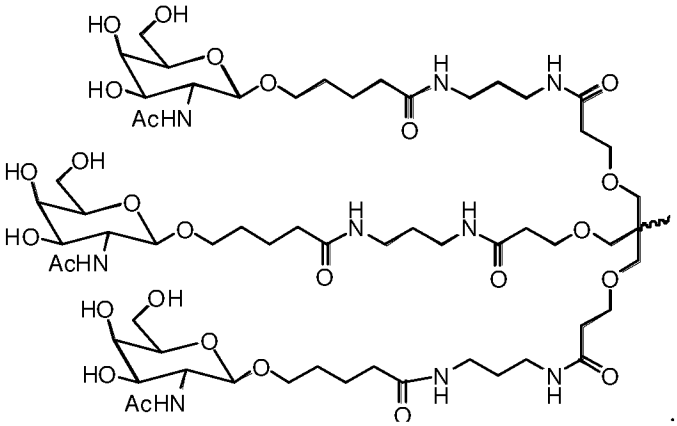
59. The dsRNA agent of any one of claims 1-58, wherein the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-deoxy, 2'-hydroxyl, and combinations thereof.

60. The dsRNA agent of claim 59, wherein the modifications on the nucleotides are 2′-O-methyl or 2′-fluoro modifications.

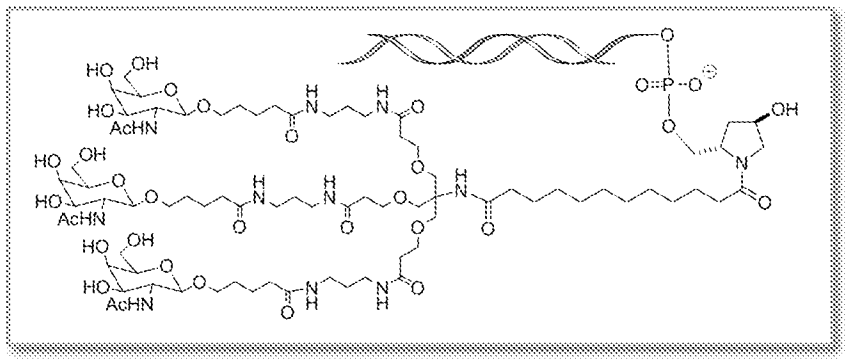
61. The dsRNA agent of any one of claims 15-30 and 40, wherein the ligand is one or more GalNAc derivatives.

62. The dsRNA agent of claim 61, wherein the ligand is attached through a bivalent or trivalent branched linker; or a cholesterol.

63. The dsRNA agent of claim 61, the ligand is



64. The dsRNA agent of claim 63, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



65. The dsRNA agent of any one of claims 1-40, wherein said agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

66. The dsRNA agent of claim 65, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand.

67. The dsRNA agent of claim 66, wherein said strand is the antisense strand.

68. The dsRNA agent of claim 66, wherein said strand is the sense strand.

69. The dsRNA agent of claim 65, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

70. The dsRNA agent of claim 69, wherein said strand is the antisense strand.

71. The dsRNA agent of claim 69, wherein said strand is the sense strand.

72. The dsRNA agent of claim 65, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand.

73. The dsRNA agent of claim 72, wherein said strand is the antisense strand.

74. The dsRNA agent of claim 40, wherein the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

75. The dsRNA agent of claim 40, wherein the Y nucleotides contain a 2′-fluoro modification.

76. The dsRNA agent of claim 40, wherein the Y′ nucleotides contain a 2′-O-methyl modification.

77. The dsRNA agent of claim 40, wherein p′>0.

78. The dsRNA agent of claim 40, wherein p′=2.

79. The dsRNA agent of claim 78, wherein q'=0, p=0, q=0, and p' overhang nucleotides are complementary to the target mRNA.

80. The dsRNA agent of claim 78, wherein q'=0, p=0, q=0, and p' overhang nucleotides are non-complementary to the target mRNA.

81. The dsRNA agent of claim 72, wherein the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

82. The dsRNA agent of any one of claims 77-81, wherein at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage.

83. The dsRNA agent of claim 82, wherein all n_p' are linked to neighboring nucleotides via phosphorothioate linkages.

84. The dsRNA agent of claim 40, wherein said dsRNA agent is selected from the group consisting of any one of the dsRNA agents listed in any one of Tables 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, and 20.

85. The dsRNA agent of claim 40, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

86. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of an insulin-like growth factor binding protein, acid labile subunit (IGFALS) gene or an insulin-like growth factor 1 (IGF-1) gene, 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 IGFALS or IGF-1, respectively, wherein each strand is about 14 to about 30 nucleotides in length, wherein said dsRNA agent is represented by formula (III):

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' (III)

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.

87. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression ofan insulin-like growth factor binding protein, acid labile subunit (IGFALS) gene or an insulin-like growth factor 1 (IGF-1) gene, 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 IGALS or IGF-1, wherein each strand is about 14 to about 30 nucleotides in length, wherein said dsRNA agent is represented by formula (III):

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' (III)

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.

88. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression ofan insulin-like growth factor binding protein, acid labile subunit (IGFALS) gene or an insulin-like growth factor 1 (IGF-1) gene, 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 IGFALS or IGF-1, respectively, wherein each strand is about 14 to about 30 nucleotides in length, wherein said dsRNA agent is represented by formula (III):

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

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 bivalent or trivalent branched linker.

89. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression ofan insulin-like growth factor binding protein, acid labile subunit (IGFALS) gene or an insulin-like growth factor 1 (IGF-1) gene, 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 IGFALS or IGF-1, respectively, wherein each strand is about 14 to about 30 nucleotides in length, wherein said dsRNA agent is represented by formula (III):

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' (III)

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 bivalent or trivalent branched linker.

90. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression ofan insulin-like growth factor binding protein, acid labile subunit (IGFALS) gene or an insulin-like growth factor 1 (IGF-1) gene, 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 IGFALS or IGF-1, respectively, wherein each strand is about 14 to about 30 nucleotides in length, wherein said dsRNA agent is represented by formula (III):

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' (IIIa)

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 bivalent or trivalent branched linker.

91. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of an insulin-like growth factor binding protein, acid labile subunit (IGFALS) gene,

wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein said 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 said 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 said sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification,

wherein said sense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus,

wherein substantially all of the nucleotides of said antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification,

wherein said antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and

wherein said sense strand is conjugated to one or more GalNAc derivatives attached through a branched bivalent or trivalent linker at the 3'-terminus.

92. The dsRNA agent of claim 91, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of nucleotides 11-62, 24-62, 79-117, 79-130, 155-173, 194-216, 194-229, 211-229, 232-293, 254-272, 310-328, 310-349, 324-345,

331-349, 353-371, 353-394, 376-394, 407-425, 439-449, 431-470, 484-515, 497-515, 541-580, 547-568, 596-647, 616-634, 673-691, 694-712, 694-734, 777-797, 781-799, 825-843, 825-855, 869-922, 958-976, 958-988, 1064-1085, 1064-1096, 1067-1085, 1067-1096, 1100-1141, 1111-1129, 1145-1163, 1145-1186, 1159-1186, 1168-1196, 1168-1214,1193-1214, 1266-1307, 1321-1339, 1342-1373, 1375-1406, 1432-1450, 1454-1472, 1519-1537, 1519-1559, 1534-1555, 1541-1559, 1606-1624, 1606-1637, 1613-1635, 1672-1690, 1672-1712, 1749-1779, 1783-1801, 1805-1823, 1806-1829, 1871-1889, 1871-1919, 1949-1977, 1993-2011, 2013-2042, 2048-2077, 2048-2088, or 2052-2084 of the nucleotide sequence of SEQ ID NO:1

93. The dsRNA agent of claim 92, wherein the antisense nucleotide sequence is selected from the group consisting of any one of the antisense nucleotide sequences of any one of duplexes AD-62728, AD-62734, AD-68111, AD-68709, AD-68712, AD-68715, AD-68716, AD-68717, AD-68719, AD-68720, AD-68722, AD-68725, AD-68726, AD-68730, AD-68731, AD-73782, AD-73773, AD-73765, AD-73946, AD-73947, AD-73858, AD-73797, AD-73808, AD-73906, AD-73912, AD-73848, AD-73836, AD-73818, AD-73786, AD-73862, AD-73795, AD-73766, AD-73930, AD-73825, AD-73924, AD-73802, AD-73767, AD-73771, AD-73777, AD-73793, AD-73898, AD-73784, AD-73882, AD-73803, AD-73772, AD-73907, AD-73948, AD-73890, AD-73883, AD-73770, AD-73867, AD-73931, AD-73932, AD-73787, AD-73791, AD-73880, AD-73914, AD-73849, AD-73863, AD-73920, AD-73944, AD-73841, AD-73785, AD-73804, AD-73823, AD-73885, AD-73788, AD-73865, AD-73941, AD-73859, AD-73913, AD-73892, AD-73837, AD-73842, AD-73840, AD-73813, AD-73796, AD-73875, AD-73900, AD-73922, AD-73861, AD-73816, AD-73764, AD-73868, AD-73812, AD-73826, AD-73938, AD-73843, AD-73817, AD-73943, AD-73827, AD-73937, AD-73877, AD-73833, AD-73807, AD-73819, AD-73886, AD-73919, AD-73800, AD-76171, AD-76173, AD-76203, AD-76210, AD-76172, AD-76175, AD-76209, AD-76174, AD-76208, AD-76186, AD-76177, AD-76199, AD-76197, and AD-76212.

94. The dsRNA agent of claim 92, wherein the sense and antisense nucleotide sequences are selected from the group consisting of any one of the sense and antisense nucleotide sequences of any one of duplexes AD-62728, AD-62734, AD-68111, AD-68709, AD-68712, AD-68715, AD-68716, AD-68717, AD-68719, AD-68720, AD-68722, AD-68725, AD-68726, AD-68730, AD-68731, AD-73782, AD-73773, AD-73765, AD-73946, AD-73947, AD-73858, AD-73797, AD-73808, AD-73906, AD-73912, AD-73848, AD-73836, AD-73818, AD-73786, AD-73862, AD-73795, AD-73766, AD-73930, AD-73825, AD-73924, AD-73802, AD-73767, AD-73771, AD-73777, AD-73793, AD-73898, AD-73784, AD-73882, AD-73803, AD-73772, AD-73907, AD-73948, AD-73890, AD-73883, AD-73770, AD-73867, AD-73931, AD-73932, AD-73787, AD-73791, AD-73880, AD-73914, AD-73849, AD-73863, AD-73920, AD-73944, AD-73841, AD-73785, AD-73804, AD-73823, AD-73885, AD-73788, AD-73865, AD-73941, AD-73859, AD-73913, AD-73892, AD-73837, AD-73842, AD-73840, AD-73813, AD-73796, AD-73875, AD-73900, AD-73922, AD-73861, AD-73816, AD-73764, AD-73868, AD-73812, AD-73826, AD-73938, AD-73843, AD-73817, AD-73943, AD-73827, AD-73937,

AD-73877, AD-73833, AD-73807, AD-73819, AD-73886, AD-73919, AD-73800, AD-76171, AD-76173, AD-76203, AD-76210, AD-76172, AD-76175, AD-76209, AD-76174, AD-76208, AD-76186, AD-76177, AD-76199, AD-76197, and AD-76212.

95. A double stranded ribonucleic acid (dsRNA) agent for inhibiting the expression of an insulin-like growth factor 1 (IGF-1) gene,

wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:11 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:12; or said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:13 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:14,

wherein substantially all of the nucleotides of said sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification,

wherein said sense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus,

wherein substantially all of the nucleotides of said antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification,

wherein said antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and

wherein said sense strand is conjugated to one or more GalNAc derivatives attached through a branched bivalent or trivalent linker at the 3'-terminus.

96. The dsRNA agent of claim 95, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of nucleotides 328-369, 340-369, 430-490, 430-482, 434-460, 532-559, 330-350, 342-362, 348-368, 349-369, 432-452, 435-455, 436-456, 438-458, 440-460, 441-461, 442-462, 449-469, 455-475, 460-480, 461-481, 462-482, 464-484, 470-490, 484-501, 534-554, 536-556, 538-558, 539-559, 542-562, 548-568, 577-597, 582-602, or 640-660 of the nucleotide sequence of SEQ ID NO:11; or at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides from any one of nucleotides 6-90, 127-145, 185-238, 247-265, 277-295, 389-417, 430-480, 543-561, 654-690, 750-768, 774-870, 894-930, 1007-1029, 1075-1126, 1144-1162, 1197-1215, 1232-1250, 1293-1311, 1334-1352, 1388-1458, 1463-1490, 1511-1529, 1599-1617, 1643-1661, 1690-1727, 1793-1825, 1843-1861, 2057-2075, 2090-2130, 2192-2228, 2310-2332, 2357-2375, 2521-2539, 2566-2588, 2648-2684, 2793-2811, 2962-2980, 3120-3142, 3208-3233, 3269-3287, 3417-3435, 3449-3467, 3575-3603, 3686-3704, 3721-3739, 3806-3824, 3939-3957, 3982-4018, 4081-4037, 4154-4172, 4271-4289, 4319-4377, 4436-4478, 4484-4502, 4523-4545, 4566-

4584, 4610-4660, 4686-4717, 4734-4769, 4780-4798, 4815-4843, 4884-4902, 4911-4929, 5004-5034, 5050-5068, 5171-5256, 5311-5364, 5409-5430, 5551-5588, 5609-5638, 5694-5712, 5715-5758, 5790-5808, 5906-5928, 5934-5952, 6323-6345, 6399-6417, 6461-6497, 6510-6535, 6584-6612, 6629-6647, 6661-6683, 6726-6789, 6796-6824, 6826-6851, 6858-6905, 6910-6927, 7004-7022, 7035-7130, 7144-7162, 7175-7241, or 7252-7270 of SEQ I DNO: 13.

97. The dsRNA agent of claim 96, wherein the antisense nucleotide sequence is selected from the group consisting of the antisense nucleotide sequence of any one of duplexes AD-66722, AD-66748, AD-66746, AD-66747, AD-66733, AD-66752, AD-66739, AD-66738, AD-66725, AD-66740, AD-66750, AD-66729, AD-66745, AD-66749, AD-66720, AD-66724, AD-66726, AD-66766, AD-66761, AD-66755, AD-66751, AD-66719, AD-66727, AD-66744, AD-66760, AD-66753, AD-66721, AD-66716, AD-66743, AD-66728, AD-77150, AD-77158, AD-74963, AD-77138, AD-75740, AD-74968, AD-74965, AD-75766, AD-75761, AD-75137, AD-74979, AD-74966, AD-75750, AD-77126, AD-74971, AD-74982, AD-77144, AD-77149, AD-75751, AD-75111, AD-77147, AD-74964, AD-74983, AD-75765, AD-74970, AD-75749, AD-77168, AD-77127, AD-75748, AD-75779, AD-75145, AD-74975, AD-77151, AD-75170, AD-75741, AD-75162, AD-74985, AD-75759, AD-75218, AD-74981, AD-75155, AD-74978, AD-77153, AD-75157, AD-75123, AD-75184, AD-77160, AD-75125, AD-75229, AD-77165, AD-75112, AD-75206, AD-75769, AD-75174, AD-75225, AD-75792, AD-75115, AD-74986, AD-77171, AD-75131, AD-77128, AD-75179, AD-75792, AD-77124, AD-75191, AD-75774, AD-75114, AD-74973, AD-77156, AD-75120, AD-75130, AD-74967, AD-75231, AD-74987, AD-77140, AD-74969, AD-75000, AD-75791, AD-75143, AD-77120, AD-77142, AD-75217, AD-75234, AD-75173, AD-75232, AD-75188, AD-75135, AD-75018, AD-77122, AD-75009, AD-75121, AD-75791, AD-77135, AD-75214, AD-74994, AD-75139, AD-75166, AD-75020, AD-77159, AD-75236, AD-77123, AD-77133, AD-74972, AD-75223, AD-75148, AD-75124, AD-75185, AD-75150, AD-74976, AD-74980, AD-75212, AD-75239, AD-75221, AD-75118, AD-75793, AD-75023, AD-75164, AD-74997, AD-74984, AD-75011, AD-75203, AD-77161, AD-75033, AD-75177, AD-75795, AD-77146, AD-75793, AD-75788, AD-75079, AD-75152, AD-77121, AD-75237, AD-75014, AD-75755, AD-75028, AD-75091, AD-75110, AD-75230, AD-75029, AD-75099, AD-77130, AD-75224, AD-75142, AD-75760, AD-75795, AD-77136, AD-75032, AD-75757, AD-75017, AD-75151, AD-75122, AD-75002, AD-75021, AD-75005, AD-75088, AD-75153, AD-75208, AD-74977, AD-75069, AD-75107, AD-74990, AD-75061, AD-75083, AD-75116, AD-75169, AD-75058, AD-74991, AD-75041, AD-77131, AD-75772, AD-77169, AD-75133, AD-75222, AD-75007, AD-75101, AD-77137, AD-75090, AD-77148, AD-75008, AD-77134, AD-74999, AD-75048, AD-75095, AD-74974, AD-75788, AD-75057, AD-75113, AD-77172, AD-75016, AD-75186, AD-75205, AD-75238, and AD-75146.

98. The dsRNA agent of claim 96, wherein the sense and antisense nucleotide sequences are selected from the group consisting of the sense and antisense nucleotide sequences of AD-66722, AD-66748, AD-66746, AD-66747, AD-66733, AD-66752, AD-66739, AD-66738, AD-66725, AD-66740,

AD-66750, AD-66729, AD-66745, AD-66749, AD-66720, AD-66724, AD-66726, AD-66766, AD-66761, AD-66755, AD-66751, AD-66719, AD-66727, AD-66744, AD-66760, AD-66753, AD-66721, AD-66716, AD-66743, and AD-66728.

99. The dsRNA agent of any one of claims 91-98, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand are modified nucleotides.

100. The dsRNA agent of any one of claims 91-98, wherein each strand has 19-30 nucleotides.

101. A cell containing the dsRNA agent of any one of claims 1-18, 40, and 87-100.

102. A pharmaceutical composition for inhibiting expression of an IGFALS gene comprising the dsRNA agent of any one of claims 1-6, 13-17, 21-37, 40-94, and 99-100.

103. A pharmaceutical composition for inhibiting expression of an IGF-1 gene comprising the dsRNA agent of any one of claims 7-14, 18-35, 38-90, and 95-100.

104. The pharmaceutical composition of claim 102 or 103, wherein the dsRNA agent is administered in an unbuffered solution.

105. The pharmaceutical composition of claim 102 or 103, wherein said dsRNA agent is administered with a buffer solution.

106. A pharmaceutical composition comprising the dsRNA agent of any one of claims 1-17, and a lipid formulation.

107. A method of inhibiting IGFALS expression in a cell, the method comprising:

contacting the cell with the dsRNA agent of any one of claims 1-6, 13-17, 21-37, 40-94, and 99-100 or a pharmaceutical composition of any one of claims 102 and 104-106; thereby inhibiting expression of the IGFALS gene in the cell.

108. A method of inhibiting IGF-1 expression in a cell, the method comprising:

contacting the cell with the dsRNA agent of any one of claims 7-14, 18-35, 38-90, and 95-100 or a pharmaceutical composition of any one of claims 103-106; thereby inhibiting expression of the IGF-1 gene in the cell.

109. The method of claim 107 or 108, wherein said cell is within a subject.

110. The method of claim 109, wherein the subject is a human.

111. The method of any one of claims 106-110, wherein IGF-1 expression is inhibited by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or to below the level of detection of the assay.

112. A method of treating a subject having a disease or disorder that would benefit from reduction in IGLAS expression or IGF-1 expression, the method comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-100 or a pharmaceutical composition of any one of claims 102-106, thereby treating said subject.

113. The method of claim 112, wherein the administration of the dsRNA to the subject causes a decrease in the IGF-1 signaling pathway.

114. The method of claim 112, wherein the disorder is an IGF system-associated disease.

115. The method of claim 114, wherein the IGF system-associated disease is acromegaly.

116. The method of claim 115 futher comprising detecting at least one sign or symptom of acromegaly in the subject.

117. The method of claim 116, wherein the at least one sign or symptom of acromegaly is selected from the group consisting of elevated IGF-1 level, somatic enlargement, excessive sweating, jaw overgrowth, sleep apnea, osteoarthropathy, joint pain, symptomatic carpal tunnel syndrome, hypertension, biventricular cardiac hypertrophy, cardiac arrhythmia, fatigue, weakness, diabetes mellitus, menstrual irregularities in women and sexual dysfunction in men, headache, visual field loss attributable to optic chiasmal compression, and diplopia due to cranial nerve palsy; in conjunction with an elevated growth hormone level.

118. The method of claim 112, wherein the IGF system-associated disease is cancer.

119. The method of claim 118 futher comprising detecting at least one sign or symptom of cancer in the subject.

120. The method of claim 119, wherein the at least one sign or symptom of cancer comprises tumor burden.

121. The method of anty one of claims 112-120, wherein the subject is human.

122. The method of any one of claims 112-121, further comprising administering an agent for the treatment of acromegaly or cancer.

123. The method of any one of claims 112-122, wherein the dsRNA agent is administered at a dose of about 0.01 mg/kg to about 50 mg/kg.

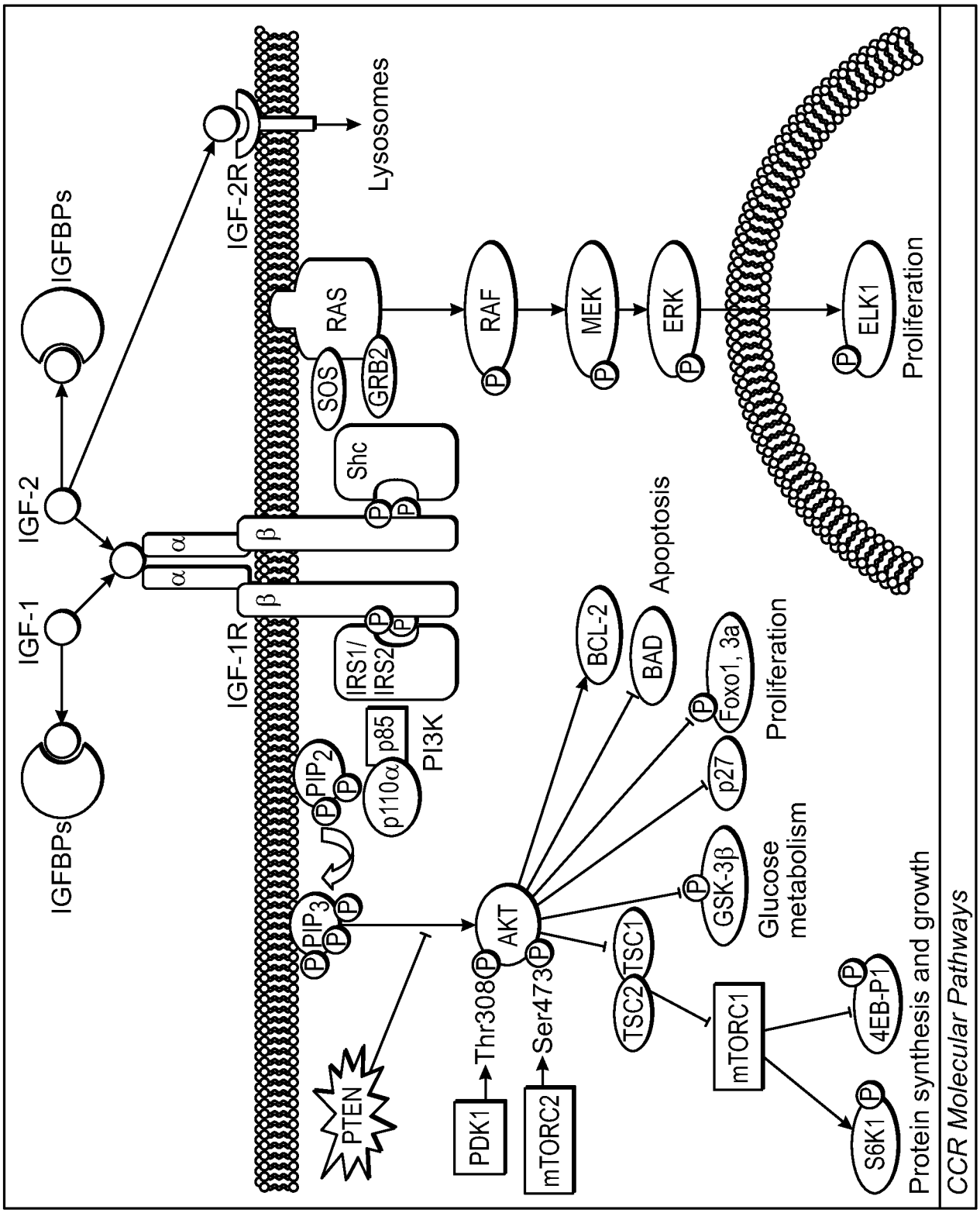
124. The method of any one of claims 112-123, wherein the dsRNA agent is administered to the subject subcutaneously.

125. The method of any one of claims 112-124, further comprising determining the level of the IGF-1 signaling pathway in said subject.

126. The method of any one of claims 112-125, further comprising determining the level of IGF-1 in said subject.

127. The method of any of claims 112-117 further comprising determining the level of growth hormone in the subject.

FIG. 1


IGF-1
IGF-2
IGFBPs
IGFBPs
IGF-2R
Lysosomes
IGF-1R
α
α
β
β
RAS
SOS
GRB2
Shc
P
P
RAF
MEK
ERK
ELK1
Proliferation
IRS1/
IRS2
p85
p110α
PI3K
PIP2
PIP3
BCL-2
BAD
Apoptosis
Foxo1, 3a
p27
Proliferation
GSK-3β
Glucose
metabolism
AKT
Thr308
Ser473
PDK1
mTORC2
PTEN
TSC1
TSC2
mTORC1
4EB-P1
S6K1
Protein synthesis and growth
CCR Molecular Pathways

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/041440

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

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

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

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

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

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

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

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/041440

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

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

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

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

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

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

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

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-6, 15-17, 36, 37, 91-94, 102, 107(completely); 13, 14, 21-35, 40-90
99-101, 104-106, 109-127(partially)

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/041440

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/712
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	C. J. AMUZIE ET AL: "Suppression of Insulin-Like Growth Factor Acid-Labile Subunit Expression--A Novel Mechanism for Deoxynivalenol-Induced Growth Retardation", TOXICOLOGICAL SCIENCES, vol. 113, no. 2, 4 October 2009 (2009-10-04), pages 412-421, XP055308785, ISSN: 1096-6080, DOI: 10.1093/toxsci/kfp225 the whole document ----- -/--	1-6, 13-17, 21-37, 40-94, 99-102, 104-107, 109-127

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 October 2016	05/12/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Romano, Alper

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LILI DU ET AL: "Hypoxia Enhances Protective Effect of Placental-Derived Mesenchymal Stem Cells on Damaged Intestinal Epithelial Cells by Promoting Secretion of Insulin-Like Growth Factor-1", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES, vol. 15, no. 2, 27 January 2014 (2014-01-27), pages 1983-2002, XP055308777, DOI: 10.3390/ijms15021983 the whole document --- KAZUO KOBAYASHI-HATTORI ET AL: "Body composition and hormonal effects following exposure to mycotoxin deoxynivalenol in the high-fat diet-induced obese mouse", MOLECULAR NUTRITION & FOOD RESEARCH, vol. 55, no. 7, 2 May 2011 (2011-05-02), pages 1070-1078, XP055308787, DE ISSN: 1613-4125, DOI: 10.1002/mnfr.201000640 figure 6 ---	1-6, 13-17, 21-37, 40-94, 99-102, 104-107, 109-127 1-3

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-6, 15-17, 36, 37, 91-94, 102, 107(completely); 13, 14, 21-35, 40-90, 99-101, 104-106, 109-127(partially)

dsRNA for inhibiting expression of IGFALS

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2. claims: 7-12, 18-20, 38, 39, 95-98, 103, 108(completely); 13, 14, 21-35, 40-90, 99-101, 104-106, 109-127(partially)

dsRNA for inhibiting expression of IGF-1
