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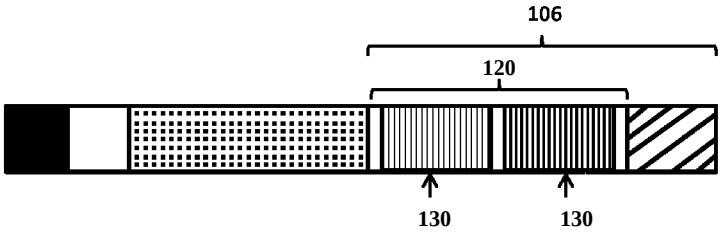
(54) Title: SIGNAL-SENSOR POLYNUCLEOTIDES FOR THE ALTERATION OF CELLULAR PHENOTYPES

FIGURE 2



(57) Abstract: The invention relates to compositions and methods for the preparation, manufacture and therapeutic use of signal-sensor polynucleotides, primary transcripts and mmRNA molecules.

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SIGNAL-SENSOR POLYNUCLEOTIDES FOR THE ALTERATION OF CELLULAR PHENOTYPES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 61,753,661, filed January 17, 2013, entitled Signal-Sensor Polynucleotides for the Alternation of Cellular Phenotypes and Microenvironments; U.S. Provisional Application No. 61/754,159, filed January 18, 2013, entitled Signal-Sensor Polynucleotides for the Alternation of Cellular Phenotypes and Microenvironments; U.S. Provisional Application No. 61/781,097, filed March 14, 2013, entitled Signal-Sensor Polynucleotides for the Alternation of Cellular Phenotypes and Microenvironments; U.S. Provisional Application No. 61/829,334, filed May 31,2013, entitled Signal-Sensor Polynucleotides for the Alternation of Cellular Phenotypes and Microenvironments; U.S. Provisional Application No. 61/839,893, filed June 27, 2013, entitled Signal-Sensor Polynucleotides for the Alternation of Cellular Phenotypes and Microenvironments; U.S. Provisional Application No. 61/842,733, filed July 3, 2013, entitled Signal-Sensor Polynucleotides for the Alternation of Cellular Phenotypes and Microenvironments; and U.S. Provisional Application No. 61/857,304, filed July 23, 2013, entitled Signal-Sensor Polynucleotides for the Alternation of Cellular Phenotypes and Microenvironments; the contents of each of which is herein incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence listing file, entitled M37PCT.txt, was created on September 30, 2013 and is 9,748,473 bytes in size. The information in electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] The invention relates to compositions, methods, processes, kits and devices for the design, preparation, manufacture and/or formulation of signal-sensor polynucleotides, primary constructs and mRNA molecules for the alteration of cellular phenotypes and microenvironments.

BACKGROUND OF THE INVENTION

[0004] Cancer is a disease characterized by uncontrolled cell division and growth within the body. In the United States, roughly a third of all women and half of all men will experience cancer in their lifetime. Polypeptides are involved in every aspect of the disease including cancer cell biology (carcinogenesis, cell cycle suppression, DNA repair and angiogenesis), treatment (immunotherapy, hormone manipulation, enzymatic inhibition), diagnosis and determination of cancer type (molecular markers for breast, prostate, colon and cervical cancer for example). With the host of undesired consequences brought about by standard treatments such as chemotherapy and radiotherapy used today, genetic therapy for the manipulation of disease-related peptides and their functions provides a more targeted approach to disease diagnosis, treatment and management.

[0005] To this end, it has been previously shown that certain modified mRNA sequences have the potential as therapeutics with benefits beyond just evading, avoiding or diminishing the immune response. Such studies are detailed in published co-pending International Publication No WO2012019168 filed August 5, 201, International Publication No WO2012045082 filed October 3, 2011, International Publication No WO2012045075 filed October 3, 2011, International Publication No WO2013052523 filed October 3, 2012, and International Publication No WO2013090648 filed December 14, 2012 the contents of which are incorporated herein by reference in their entirety.

[0006] The use of modified polynucleotides in the fields of antibodies, viruses, veterinary applications and a variety of in vivo settings have been explored and are disclosed in, for example, co-pending and co-owned U.S. Provisional Patent Application No 61/618,862, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Biologies; U.S. Provisional Patent Application No 61/681,645, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Biologies; U.S. Provisional Patent Application No 61/737,130, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Biologies; U.S. Provisional Patent Application No 61/618,866, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Antibodies; U.S. Provisional Patent Application No 61/681,647, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Antibodies; U.S. Provisional

Patent Application No 61/737,134, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Antibodies; U.S. Provisional Patent Application No 61/618,868, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Vaccines; U.S. Provisional Patent Application No 61/681,648, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Vaccines; U.S. Provisional Patent Application No 61/737,135, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Vaccines; U.S. Provisional Patent Application No 61/618,870, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides; U.S. Provisional Patent Application No 61/681,649, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides; U.S. Provisional Patent Application No 61/737,139, filed December 14, 2012, Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides; U.S. Provisional Patent Application No 61/618,873, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins; U.S. Provisional Patent Application No 61/681,650, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins; U.S. Provisional Patent Application No 61/737,147, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins; U.S. Provisional Patent Application No 61/618,878, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins; U.S. Provisional Patent Application No 61/681,654, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins; U.S. Provisional Patent Application No 61/737,152, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins; U.S. Provisional Patent Application No 61/618,885, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; U.S. Provisional Patent Application No 61/681,658, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; U.S. Provisional Patent Application No 61/737,155, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; U.S. Provisional Patent Application No 61/618,896, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins; U.S.

Provisional Patent Application No 61/668,157, filed July 5, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins; U.S. Provisional Patent Application No 61/681,661, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins; U.S. Provisional Patent Application No 61/737,160, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins; U.S. Provisional Patent Application No 61/618,91 1, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins; U.S. Provisional Patent Application No 61/681,667, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins; U.S. Provisional Patent Application No 61/737,168, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins; U.S. Provisional Patent Application No 61/618,922, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins; U.S. Provisional Patent Application No 61/681,675, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins; U.S. Provisional Patent Application No 61/737,174, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins; U.S. Provisional Patent Application No 61/618,935, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/681,687, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/737,184, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/618,945, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/681,696, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/737,191, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/618,953, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/681,704, filed August 10, 2012, entitled Modified

Polynucleotides for the Production of Proteins Associated with Human Disease; U.S. Provisional Patent Application No 61/737,203, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; US Provisional Patent Application No 61/681,720, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Cosmetic Proteins and Peptides; US Provisional Patent Application No 61/737,213 , filed December 14, 2012, entitled Modified Polynucleotides for the Production of Cosmetic Proteins and Peptides; US Provisional Patent Application No. 61/681,742, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Oncology-Related Proteins and Peptides; International Application No PCT/US2013/030062, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Biologies and Proteins Associated with Human Disease; US Patent Application No 13/791,922, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Biologies and Proteins Associated with Human Disease; International Application No PCT/US2013/030063, filed March 9, 2013, entitled Modified Polynucleotides; International Application No. PCT/US20 13/030064, entitled Modified Polynucleotides for the Production of Secreted Proteins; US Patent Application No 13/791,921, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Secreted Proteins; International Application No PCT/US20 13/030059, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Membrane Proteins; International Application No. PCT/US20 13/030066, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins; International Application No. PCT/US20 13/030067, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Nuclear Proteins; International Application No. PCT/US20 13/030060, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Proteins; International Application No. PCT/US20 13/030061, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; US Patent Application No 13/791,910, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease; International Application No. PCT/US20 13/030068, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Cosmetic Proteins and Peptides; and International Application No.

PCT/US20 13/030070, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Oncology-Related Proteins and Peptides; International Patent Application No. PCT/US20 13/03 1821, filed March 15, 2013, entitled In Vivo Production of Proteins; the contents of each of which are herein incorporated by reference in their entireties.

[0007] Formulations and delivery of modified polynucleotides are described in, for example, co-pending and co-owned International Publication No WO20 13090648, filed December 14, 2012, entitled Modified Nucleoside, Nucleotide, Nucleic Acid Compositions and US Publication No US20130156849, filed December 14, 2012, entitled Modified Nucleoside, Nucleotide, Nucleic Acid Compositions; the contents of each of which are herein incorporated by reference in their entireties.

[0008] The next generation of therapeutics must also address the complex cellular microenvironment of the cancer and have the capacity for cell, tissue, organ or patient stratification, whether structurally or functionally.

[0009] The present invention addresses this need by providing nucleic acid based compounds or polynucleotide-encoding nucleic acid-based compounds (e.g., signal-sensor polynucleotides) which encode a polypeptide of interest and which have structural and/or chemical features that allow for greater selectivity, profiling or stratification along defineable disease characteristics or metrics.

SUMMARY OF THE INVENTION

[0010] Described herein are compositions, methods, processes, kits and devices for the design, preparation, manufacture and/or formulation of signal-sensor polynucleotide molecules encoding at least one oncology-related polypeptide of interest. Such signal-sensor polynucleotides may be chemically modified mRNA (mmRNA) molecules.

[0011] The present invention provides an isolated signal-sensor polynucleotide comprising a region encoding an oncology-related polypeptide of interest that functions, when translated, to send a death or survival signal. Such death or survival signals include those which (i) alter (increase or decrease) the expression of one or more proteins, nucleic acids, or non-coding nucleic acids, (ii) alter the binding properties of biomolecules within the cell, and/or (iii) perturb the cellular microenvironment in a therapeutically benificial way.

[0012] Optionally, the signal-sensor polnucleotide may also encode in a flanking region, one or more sensor sequences. Such sensor sequences function to "sense" the cell, tissue or ogan microenvironment and confer upon the signal-sensor polynucleotide an altered expression or half life profile (increased or decreased) depending on the interactions of the sensor sequence with the cell, tissue or organ microenvironment.

[0013] In one aspect, provided herein are signal -sensor polynucleotide comprising, a first region of linked nucleosides, a first flanking region located 5' relative to said first region and a second flanking region located 3' relative to said first region. The first region may encode an oncology-related polypeptide of interest such as, but not limited to, SEQ ID NOs: 1321-2487, 661 1-6616 and 7355-7361, 7490, 7492, 7493, 7512, 7514, 7516 and 7517 and the first flanking region may include a sequence of linked nucleosides such as, but not limited to, the native 5' untranslated region (UTR) of any of the nucleic acids that encode any of SEQ ID NOs: 1321-2487, 661 1-6616, 7355-7361, 7490, 7492, 7493, 7512, 7514, 7516, 7517, SEQ ID NO: 1-4 and functional variants thereof. The first region may comprise at least an open reading frame of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2488-2496, 6617-6621, 7348-7354, 7362-7489, 7491, 7494, 7506, 751 1 and 7513.

[0014] The second flanking region may include a sequence of linked nucleosides such as, but not limited to, the native 3' UTR of any of the nucleic acids that encode any of SEQ ID NOs: 1321-2487, 661 1-6616, 7355-7361, 7490, 7492, 7493, 7512, 7514, 7516, 7517, SEQ ID NO: 5-21 and functional variants thereof, and one or more sensor sequences located such as, but not limited to, SEQ ID NOs: 3529-4549, SEQ ID NOs: 5571-6591 and functional variants thereof. The signal-sensor polynucleotide may also include a 3' tailing sequence of linked nucleosides.

[0015] In another aspect, provided herein is a signal-sensor polynucleotide which comprises an mRNA encoding an oncology-related polypeptide of interest and one or more sensor sequences such as, but not limited to, SEQ ID NOs: 3529-4549, SEQ ID NOs: 5571-6591 and functional variants thereof. The oncology-related polypeptide of interest may be, but is not limited to, SEQ ID NOs: 1321-2487, 661 1-6616, 7355-7361, 7490, 7492, 7493, 7512, 7514, 7516 and 7517. The mRNA may include at least one open reading frame of a nucleic acid sequence selected from the group consisting of

SEQ ID NOs: 2488-2496, 6617-6621, 7348-7354, 7362-7489, 7491, 7494, 7506, 751 1 and 7513.

[0016] The signal-sensor polynucleotides may comprise one, two, three or more than three stop codons. In one aspect, the signal-sensor polynucleotides comprise two stop codons. As a non-limiting example, the first stop codon is "TGA" and the second stop codon is selected from the group consisting of "TAA," "TGA" and "TAG." In another aspect, signal-sensor polynucleotides comprise three stop codons.

[0017] The signal-sensor polynucleotides may have a 3' tailing sequence of linked nucleosides such as, but not limited to, a poly-A tail of at least 140 nucleotides, a triple helix, and a poly A-G quartet.

[0018] The signal-sensor polynucleotides may have a 5'cap such as, but not limited to, CapO, Capl, ARCA, inosine, Nl-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

[0019] In one aspect, the signal-sensor polynucleotides may include at least one chemical modification such as, but not limited to, modifications located on one or more of a nucleoside and/or the backbone of the nucleotides. In one embodiment, the signal-sensor polynucleotides comprise a pseudouridine analog such as, but not limited to, 1-carboxymethyl-pseudouridine, 1-propynyl-pseudouridine, 1-taurinomethyl-pseudouridine, l-taurinomethyl-4-thio-pseudouridine, 1-methyl-pseudouridine (m^), 1-methyl-4-thio-pseudouridine (m^1sV), 4-thio-l-methyl-pseudouridine, 3-methyl-pseudouridine ($m^3\psi$), 2-thio-l-methyl-pseudouridine, 1-methyl- 1-deaza-pseudouridine, 2-thio- 1-methyl- 1-deaza-pseudouridine, dihydropseudouridine, 2-thio-dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, Nl-methyl-pseudouridine, l-methyl-3-(3-amino-3-carboxypropyl)pseudouridine ($acp^3\psi$), and 2'-0-methyl-pseudouridine (ψm). In another embodiment, the signal-sensor polynucleotides comprise the pseudouridine analog 1-methylpseudouridine. In yet another embodiment, the signal-sensor polynucleotides comprise the pseudouridine analog 1-methylpseudouridine and the modified nucleoside 5-methylcytidine.

[0020] In another aspect, the signal sensor-polynucleotides may include at least two chemical modifications such as, but not limited to, modifications located on one or more of a nucleoside and/or the backbone of the nucleotides. As a non-limiting example, the signal-sensor polynucleotide comprises the chemical modifications 1-methylpseudouridine and 5-methylcytidine.

[0021] The signal-sensor polynucleotides may comprise at least one translation enhancer element (TEE) such as, but not limited to, TEE-001 - TEE-705.

[0022] In one aspect, the signal-sensor polynucleotide encodes a factor modulating the affinity between HIF subunits and/or HIF-dependent gene expression such as, but not limited to, SEQ ID NO: 661 1-6616.

[0023] The signal-sensor polynucleotides may be purified and/or formulated.

[0024] Employing the signal-sensor polynucleotides, the present invention provides a method of treating a disease, disorder and/or condition in a subject in need thereof by increasing the level of an oncology-related polypeptide of interest comprising administering to said subject an isolated signal-sensor polynucleotide encoding said oncology-related polypeptide. The disease, disorder and/or condition may include, but is not limited to, adrenal cortical cancer, advanced cancer, anal cancer, aplastic anemia, bileduct cancer, bladder cancer, bone cancer, bone metastasis, brain tumors, brain cancer, breast cancer, childhood cancer, cancer of unknown primary origin, Castleman disease, cervical cancer, colon/rectal cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, gestational trophoblastic disease, Hodgkin disease, Kaposi sarcoma, renal cell carcinoma, laryngeal and hypopharyngeal cancer, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myelomonocytic leukemia, liver cancer, non-small cell lung cancer, small cell lung cancer, lung carcinoid tumor, lymphoma of the skin, malignant mesothelioma, multiple myeloma, myelodysplasia syndrome, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma in adult soft tissue, basal and

squamous cell skin cancer, melanoma, small intestine cancer, stomach cancer, testicular cancer, throat cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, Wilms tumor and secondary cancers caused by cancer treatment.

[0025] The present invention provides a method of reducing, eliminating,or preventing tumor growth in a subject in need thereof by increasing the level of an oncology-related polypeptide of interest comprising administering to said subject an isolated signal-sensor polynucleotide encoding said oncology-related polypeptide. The tumor growth may be associated with or results from a disease, disorder and/or condition such as, but not limited to, adrenal cortical cancer, advanced cancer, anal cancer, aplastic anemia, bileduct cancer, bladder cancer, bone cancer, bone metastasis, brain tumors, brain cancer, breast cancer, childhood cancer, cancer of unknown primary origin, Castleman disease, cervical cancer, colon/rectal cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, gestational trophoblastic disease, Hodgkin disease, Kaposi sarcoma, renal cell carcinoma, laryngeal and hypopharyngeal cancer, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myelomonocytic leukemia, liver cancer, non-small cell lung cancer, small cell lung cancer, lung carcinoid tumor, lymphoma of the skin, malignant mesothelioma, multiple myeloma, myelodysplasia syndrome, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma in adult soft tissue, basal and squamous cell skin cancer, melanoma, small intestine cancer, stomach cancer, testicular cancer, throat cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, Wilms tumor and secondary cancers caused by cancer treatment.

[0026] The present invention provides a method of reducing and/or ameliorating at least one symptom of cancer in a subject in need thereof by increasing the level of a polypeptide of interest comprising administering to said subject an isolated signal-sensor

polynucleotide encoding said oncology-related polypeptide. Non-limiting examples of symptoms include weakness, aches and pains, fever, fatigue, weight loss, blood clots, increased blood calcium levels, low white blood cell count, short of breath, dizziness, headaches, hyperpigmentation, jaundice, erthema, pruritis, excessive hair growth, change in bowel habits, change in bladder function, long-lasting sores, white patches inside the mouth, white spots on the tongue, unusual bleeding or discharge, thickening or lump on parts of the body, indigestion, trouble swallowing, changes in warts or moles, change in new skin and nagging cough and hoarseness.

[0027] The present invention provides a method of preferentially inducing cell death in cancer cells in a tissue or organ comprising contacting the tissue or organ with a signal-sensor polynucleotide encoding an oncology-related polypeptide whose expression triggers apoptosis or cell death and at least one microRNA binding site of a microRNA where the expression of the microRNA in the cancer cell is lower than the expression of the mircroRNA in normal non-cancerous cells.

[0028] The signal-sensor polynucleotide may be administered at a total daily dose of between 0.001 ug and 150 ug. Administration of a signal-sensor polynucleotide may be by injection, topical administration, ophthalmic administration or intranasal administration. In one aspect, administration may be by injection such as, but not limited to, intradermal, subcutaneous and intramuscular. In another aspect, administration may be topical such as, but not limited to, using creams, lotions, ointments, gels, sprays, solutions and the like.

[0029] The details of various embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and the drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] The foregoing and other objects, features and advantages will be apparent from the following description of particular embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of various embodiments of the invention.

[0031] FIG. 1 is a schematic of a primary construct of the present invention.

[0032] FIG. 2 is an expanded schematic of the second flanking region of a primary construct of the present invention illustrating the signal-sensor elements of the polynucleotide.

[0033] FIG. 3 is a gel profile of Apoptosis-Inducing Factor short (AIFsh) protein from AIFsh modified mRNA in mammals. Figure 3A shows the expected size of AIFsh. Figure 3B shows the expected size of AIFsh.

[0034] FIG. 4 is a gel profile of Siah E3 ubiquitin protein ligase 1 (SIAH1) protein from SIAH1 modified mRNA in mammals. Figure 4A shows the expected size of SIAH1. Figure 4B shows the expected size of SIAH1.

[0035] FIG. 5 is a gel profile of constitutively active (C.A.) caspase 3 (also known as reverse caspase 3 (Rev-Caspase 3)) protein from C.A. caspase 3 modified mRNA in mammals. Figure 5A shows the expected size of C.A. caspase 3. Figure 5B shows the expected size of C.A. caspase 3.

[0036] FIG. 6 is a gel profile of Granulysin protein from granulysin modified mRNA in mammals. Figure 6A shows the expected size of granulysin. Figure 6B shows the expected size of granulysin.

[0037] FIG. 7 is a western blot of C.A. caspase 3 and C.A. caspase 6. Figure 7A shows protein from C.A. caspase 3 modified mRNA fully modified with 5-methylcytidine and 1-methylpseudouridine or fully modified with 1-methylpseudouridine. Figure 7B shows protein from C.A. caspase 6 modified mRNA fully modified with 5-methylcytidine and 1-methylpseudouridine or fully modified with 1-methylpseudouridine.

DETAILED DESCRIPTION

[0038] It is of great interest in the fields of therapeutics, diagnostics, reagents and for biological assays to be able to deliver a nucleic acid, e.g., a ribonucleic acid (RNA) inside a cell, whether *in vitro, in vivo, in situ* or *ex vivo,* such as to cause intracellular translation of the nucleic acid and production of an encoded polypeptide of interest. Of particular importance is the delivery and function of a non-integrative polynucleotide.

[0039] Described herein are compositions (including pharmaceutical compositions) and methods for the design, preparation, manufacture and/or formulation of polynucleotides encoding one or more polypeptides of interest. Also provided are systems, processes,

devices and kits for the selection, design and/or utilization of the polynucleotides encoding the polypeptides of interest described herein.

[0040] To this end, polypeptides of the present invention are encoded by a new class of polynucleotide therapeutics, termed "signal-sensor polynucleotides" which are particularly useful in the stratification, profiling and/or personalization of the polynucleotide therapeutice (e.g., mRNA) and which are tailored to a particular cell type, disease or cell microenvironment or biological profile.

[0041] It is known that cancers exhibit diverse gene expression patterns, physicochemical environments and metastatic or motility behaviors and according to Hanahan and Weinberg (Cell, 201 1, 144:646-674) there are six hallmarks of cancer. These include sustaining a proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. These hallmarks or functions of cancer allow the cancer to survive, proliferate and disseminate and each arises at different times and in different patterns depending on the cancer type.

[0042] The development of cancer therapeutics which to selectively target the cancer cells while sparing normal cells dominates ongoing efforts in every area of oncology. The polynucleotides of the present invention represent such therapeutics; having the ability to selectively stabilize or destabilize cell systems, signal proliferation (survival) or death, trigger the cell cycle or senescence and/or activate or avoid the immune response depending on the cell type, e.g., cancer or normal cell.

[0043] According to the present invention, signal-sensor polynucleotide therapeutics may be used to destabilize the survival advantages or hallmarks of a cancer cell (hence they would be cytotoxic). In one embodiment diagnostic efforts would include the profiling of the cancer (although this would not be required *apriori)* including including metabolic state (hypoxic, acidotic), apoptotic vs. survival gene profiles, cell cycle vs. senescent stage, immune status, and stromal factors present.

[0044] In one embodiment the signal-sensor polynucleotide disrupts the transcriptome of the cancer cell. The disruption may affect one or more signaling or expression events. For example the encoded oncology-related polypeptide may act upstream of a transcription factor known to induce or enhance the expression of genes associated with a

cancer. Delivery of the signal-sensor polynucleotide encoding the oncology-related polypeptide which inhibits such a transcription factor (either by binding or sequestration or degradation) would thereby alter the transcriptome of the cancer cell and have a therapeutic benefit. One such transcription factor is HIF-1 alpha. A signal-sensor polynucleotide encoding a protein which is capable of binding HIF-1 alpha or whose expression results in lower HIF-1 alpha, would effectively turn down HIF-1 alpha regulated genes, e.g., VEGFA or SLC2A1, and destabilize the cancer.

[0045] In one embodiment, the profile of the cancer may be evaluated before the signal-sensor polynucleotide is selected. Such profiling data would inform the selection of which oncology-related polypeptide to be delivered. The profile of gene expression, categorized by hallmark class such as apoptosis, replicative capacity or metabolic signature would allow dynamic instability scoring for a polypeptide and an optimization of therapeutic window for the signal-sensor polynucleotide. As used herein, a "dynamic instability index" refers to a dose of signal-sensor polynuclotide sufficient to induce 50% increase of the oncology-related target protein *in vitro* in a cancer cell as compared to a normal matched cell.

[0046] Profiling may also be done within hallmark classes such as the distinction between caspase-dependent and caspase independent gene expression for the apoptosis class. Alternatively, profiling could be conducted across classes such as gene profiling of apoptosis, senescence (replicative capacity), and metabolic classes.

[0047] In one embodiment, the signal-sensor polynucleotides described herein may be used to reduce the expression and/or amount of a polypeptide in a cell. As a non-limiting example, MYC inhibitor A, MYC inhibitor B, MYC inhibitor C or MYC inhibitor D may be used on Hep3B cells in order to determine the potentcy of MYC inhibitor A, MYC inhibitor B, MYC inhibitor C or MYC inhibitor D at various concentrations (see e.g., Example 55).

[0048] In one embodiment, the signal-sensor polynucleotides described hereim may direct either cytotoxic or cytoprotective therapeutic benefit to specific cells, e.g., normal vs. cancerous.

[0049] In one embodiment signal-sensor polynucleotides would not only encode an oncology-related polypeptide but also a sensor sequence. Sensor sequences include, for

example, microRNA binding sites, transcription factor binding sites, artificial binding sites engineered to act as pseudo-receptors for endogenous nucleic acid binding molecules. A "sensor region" is a region of linked nucleosides of the signal-sensor polynucleotide comprising at least one sensor sequence. The signal-sensor polynucleotides of the present invention may have one or more sensor regions.

[0050] In one embodiment, one or more sensor regions may be located in the first flanking region. As a non-limiting example, the sensor region in the first flanking region may comprise at least one sensor sequence. The sensor sequence may be, but is not limited to, mir-122, mir-142-3p, mir-142-5p, mir-146, fragments or variants thereof. As another non-limiting example, the sensor region in the first flanking region may comprise at least one sensor sequence such as a mir-122 sequence. The mir-122 sequence may be, but is not limited to, a mir-122 binding site, mir-122 seed sequence, mir-122 binding site without the seed sequence or a combination thereof.

[0051] In another embodiment, one or more sensor regions may be located in the second flanking region. As a non-limiting example, the sensor region in the second flanking region may include a sensor sequence such as mir-122, mir-142-3p, mir-142-5p, mir-146, fragments or variants thereof. As another non-limiting example, the sensor region in the second flanking region may include three sensor sequences. The sensor sequences may be, but are not limited to, mir-122 sequences such as mir-122 binding sites, mir-122 seed sequences, mir-122 binding sites without the seed sequence or a combination thereof. As yet another non-limiting example, the sensor region in the second flanking region is located in the 3'UTR and the sensor region may include a sensor sequence which is a mir-122 sequence. The mir-122 sequence may be, but is not limited to, a mir-122 binding site, mir-122 seed sequence, mir-122 binding site without the seed sequence or a combination thereof.

[0052] In one embodiment, two or more sensor regions may be located in the same region of the signal-sensor polynucleotide such as, but not limited to, a first region first region of linked nucleotides, the first flanking region and/or the second flanking region. As a non-limiting example, the two or more sensor regions are located in the second flanking region. As yet another non-limiting example, three sensor regions are located in the 3' UTR in the second flanking region. The three sensor regions may include, mir-122

binding sites, mir-122 seed sequences, mir-122 binding sites without the seed sequence or a combination thereof

[0053] In another embodiment, two or more sensor regions may be located in different regions of the signal-sensor polynucleotide such as, but not limited to, the first region of linked nucleotides, the first flanking region and/or the second flanking region. As a non-limiting example, a first sensor region is located in the first flanking region and a second sensor region is located in the second flanking region. The sensor regions may comprise the same sensor sequence or different sensor sequences.

[0054] In one embodiment, a start codon is located within a sensor region.

[0055] In one embodiment, a sensor region may comprise two or more sensor sequences. The sensor sequences may be the same or different.

[0056] In one embodiment, the sensor region may comprise two or more sensor sequence which are different from each other but they may be based on the same mir binding site. As a non-limiting example, the sensor region may include at least one miR binding site sequence and at least one mir binding site sequence with the seed removed. As another non-limiting example, the sensor region may include at least one miR binding site sequence and at least one miR seed sequence. As yet another non-limiting example, the sensor region may include at least one miR binding site sequence with the seed removed and at least one miR seed sequence.

[0057] In another embodiment, the sensor region may comprise two or more sensor sequences which are in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times. In these patterns, each letter, A, B, or C represent a different miR sequence.

[0058] In yet another embodiment, the signal-sensor polynucleotide may include two or more sensor regions with each sensor region having one or more sensor sequences. As a non-limiting example, the sensor sequences may be in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times in each of the sensor regions. As another non-limiting example, the sensor sequences may be in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times across the entire signal-sensor polynucleotide. In these patterns, each letter, A, B, or C represent

a different miR sequence. As a non-limiting example, the first sensor region may have sensor sequences in the pattern ABA and the second sensor region may have sensor sequences in the pattern BAB so the overall pattern of the sensor sequences in the signal-sensor polynucleotide is ABABAB. As another non-limiting example, the first sensor region may have sensor sequences AA, the second sensor region may have sensor sequences BB, the third sensor region may have sensor sequences AA and the fourth sensor region may have sensor seuqences BB so the overall pattern of the sensor sequences in the signal-sensor polynucleotide is AABBAABB.

[0059] The sensor sequences in the signal-sensor polynucleotides of the present invention may include one or more regulatory sequences in the 3-UTR and/or 5'UTR of natural mRNAs, which regulate mRNA stability and translation in different tissues and cells. Such cis-regulatory elements may include, but are not limited to, Cis- RNP (Ribonucleoprotein)/RBP (RNA binding protein) regulatory elements, AU-rich element AUE, structured stem-loop, constitutive decay elements (CDEs), GC-richness and other structured mRNA motifs (Parker BJ et al, Genome Research, 201 1, 21, 1929-1943, which is herein incorporated by reference in its entirety.). For example, CDEs are a class of regulatory motifs that mediate mRNA degradation through their interaction with Roquin proteins. In particular, CDEs are found in many mRNAs that encode regulators of development and inflammation to limit cytokine production in macrophage (Leppek K et al, Cell, 2013, 153, 869-881, which is herein incorporated by reference in its entirety.).

[0060] In one embodiment, a particular CDE can be introduced to the signal-sensor polynucleotide when the degradation of polypeptides in a cell or tissue is desired. A particular CDE can also be removed from the signal-sensor polynucleotide in order to maintain a more stable mRNA in a cell or tissue for sustaining protein expression.

[0061] In one embodiment, microRNA (miRNA) profiling of the cancer cells or tissues may be conducted to determine the presence or absence of miRNA in the cells or tissues to determine the appropriate microRNA to use as sensor sequences in the signal sensor polynucleotides.

[0062] MicroRNA gene regulation may be influenced by the sequence surrounding the microRNA such as, but not limited to, the species of the surrounding sequence, the type of sequence (e.g., heterologous, homologous and artificial), regulatory elements in the

surrounding sequence and/or structural elements in the surrounding sequence. The microRNA may be influenced by the 5'UTR and/or the 3'UTR. As a non-limiting example, a non-human 3'UTR may increase the regulatory effect of the microRNA sequence on the expression of a polypeptide of interest compared to a human 3'UTR of the same sequence type.

[0063] Other regulatory elements and/or structural elements of the 5'-UTR can influence microRNA mediated gene regulation. One such example is a structured IRES (Internal Ribosome Entry Site) in the 5'UTR, which is necessary for the binding of translational elongation factors to initiate protein translation. EIF4A2 binding to this secondarily structured element in the 5'UTR is necessary for microRNA mediated gene expression (Meijer HA et al, Science, 2013, 340, 82-85, herein incorporated by reference in its entirety). The sensor-signal polynucleotide can further be modified to include this structured 5'-UTR in order to enhance microRNA mediated gene regulation.

[0064] At least one microRNA site can be engineered into the 3' UTR of the signal-sensor polynucleotides of the present invention. In this context, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten or more microRNA sites may be engineered into the 3' UTR of the signal-sensor polynucleotides of the present invention. In one embodiment, the microRNA sites incorporated into the signal-sensor polynucleotides may be the same or may be different microRNA sites. In another embodiment, the microRNA sites incorporated into the signal-sensor polynucleotides may target the same or different tissues in the body. As a non-limiting example, through the introduction of tissue-, cell-type-,or disease-specific microRNA binding sites in the 3' UTR of a signal-sensor polynucleotide, the degree of expression in specific cell types (e.g. hepatocytes, myeloid cells, endothelial cells, cancer cells, etc.) can be reduced.

[0065] In one embodiment, a microRNA site can be engineered near the 5' terminus of the 3'UTR, about halfway between the 5' terminus and 3'terminus of the 3'UTR and/or near the 3'terminus of the 3'UTR. As a non-limiting example, a microRNA site may be engineered near the 5' terminus of the 3'UTR and about halfway between the 5' terminus and 3'terminus of the 3'UTR. As another non-limiting example, a microRNA site may be engineered near the 3'terminus of the 3'UTR and about halfway between the

5' terminus and 3'terminus of the 3'UTR. As yet another non-limiting example, a microRNA site may be engineered near the 5' terminus of the 3'UTR and near the 3' terminus of the 3'UTR.

[0066] In another embodiment, a 3'UTR can comprise 4 microRNA sites. The microRNA sites may be complete microRNA binding sites, microRNA seed sequences and/or microRNA binding site sequences without the seed sequence.

[0067] In one embodiment, a signal-sensor polynucleotide may be engineered to include microRNA sites which are expressed in different tissues of a subject. As a non-limiting example, a signal-sensor polynucleotide of the present invention may be engineered to include miR-192 and miR-122 to regulate expression of the signal-sensor polynucleotide in the liver and kidneys of a subject. In another embodiment, a signal-sensor polynucleotide may be engineered to include more than one microRNA sites for the same tissue. For example a signal-sensor polynucleotide of the present invention may be engineered to include miR- 17-92 and miR-126 to regulate expression of the signal-sensor polynucleotide in endothelial cells of a subject.

[0068] In one embodiment, the therapeutic window and or differential expression associated with the oncology-related polypeptide encoded by the signal-sensor polynucleotide of the invention may be altered. For example, signal-sensor polynucleotides may be designed whereby a death signal is more highly expressed in cancer cells (or a survival signal in a normal cell) by virtue of the miRNA signature of those cells. Where a cancer cell expresses a lower level of a particular miRNA, the signal-sensor polynucleotide encoding the binding site for that miRNA (or miRNAs) would be more highly expressed. Hence, the oncology-related polypeptide encoded by the signal-sensor polynucleotide is selected as a protein which triggers or induces cell death. Neigboring noncancer cells, harboring a higher expression of the same miRNA would be less affected by the encoded death signal as the signal-sensor polynucleotide would be expressed at a lower level due to the affects of the miRNA binding to the binding site or "sensor" encoded in the 3'UTR. Conversely, cell survival or cytoprotective signals may be delivered to tissues containing cancer and non cancerous cells where a miRNA has a higher expression in the cancer cells—the result being a lower survival signal to the cancer cell and a larger survival signature to the normal cell.

Multiple signal-sensor polynucleotides may be designed and administered having different signals according to the previous paradigm.

[0069] In one embodiment, the expression of a signal-sensor polynucleotide may be controlled by incorporating at least one sensor sequence in the signal-sensor polynucleotide and formulating the signal-sensor polynucleotide. As a non-limiting example, a polynucleotide may be targeted to an orthotopic tumor by having a polynucleotide incorporating a miR-122 binding site and formulated in a lipid nanoparticle comprising the cationic lipid DLin-KC2-DMA (see e.g., the experiments described in Example 56A and 56B).

[0070] Through an understanding of the expression patterns of microRNA in different cell types, signal-sensor polynucleotides can be engineered for more targeted expression in specific cell types or only under specific biological conditions. Through introduction of tissue-specific microRNA binding sites, signal-sensor polynucleotides could be designed that would be optimal for protein expression in a tissue or in the context of a biological condition such as cancer.

[0071] Transfection experiments can be conducted in relevant cell lines, using engineered signal-sensor polynucleotides and protein production can be assayed at various time points post-transfection. For example, cells can be transfected with different microRNA binding site-engineering nucleic acids or signal-sensor polynucleotides and by using an ELISA kit to the relevant protein and assaying protein produced at 6 hr, 12 hr, 24 hr, 48 hr, 72 hr and 7 days post-transfection. *In vivo* experiments can also be conducted using microRNA-binding site-engineered molecules to examine changes in tissue-specific expression of formulated signal-sensor polynucleotides.

[0072] In one embodiment, the signal-sensor polynucleotides of the invention may include at least one microRNA in order to dampen the antigen presentation by antigen presenting cells. The microRNA may be the complete microRNA sequence, the microRNA seed sequence, the microRNA sequence without the seed or a combination thereof. As a non-limiting example, the microRNA incorporated into the signal-sensor polynucleotide may be specific to the hematopoietic system. As another non-limiting example, the microRNA incorporated into the signal-sensor polynucleotides of the invention to dampen antigen presentation is miR-142-3p.

[0073] In one embodiment, the signal-sensor polynucleotides of the invention may include at least one microRNA in order to dampen expression of the encoded polypeptide in a cell of interest. As a non-limiting example, the signal-sensor polynucleotides of the invention may include at least one miR-122 binding site in order to dampen expression of an encoded polypeptide of interest in the liver. As another non-limiting example, the signal-sensor polynucleotides of the invention may include at least one miR-142-3p binding site, miR-142-3p seed sequence, miR-142-3p binding site without the seed, miR-142-5p binding site, miR-142-5p seed sequence, miR-142-5p binding site without the seed, miR-146 binding site, miR-146 seed sequence and/or miR-146 binding site without the seed sequence (see e.g., the experiment outlined in Example 47 and Example 60).

[0074] According to the present invention, the signal-sensor polynucleotides described herein may be modified as to avoid the deficiencies of other polypeptide-encoding molecules of the art. Hence, in this embodiment the signal-sensor polynucleotides are referred to as modified signal-sensor polynucleotides or primary constructs, modified mRNA or mmRNA.

[0075] Provided herein, in part, are signal-sensor polynucleotide polynucleotides, primary constructs and/or mmRNA encoding oncology-related polypeptides of interest which have been designed to improve one or more of the stability and/or clearance in tissues, receptor uptake and/or kinetics, cellular access by the compositions, engagement with translational machinery, mRNA half-life, translation efficiency, immune evasion, protein production capacity, secretion efficiency (when applicable), accessibility to circulation, protein half-life and/or modulation of a cell's status, function and/or activity.

I. Compositions of the Invention

[0076] The present invention provides nucleic acid molecules, specifically signal-sensor polynucleotides, primary constructs and/or mmRNA which encode one or more oncology-related polypeptides of interest. Specifically the invention contemplates signal-sensor polynucleotides which are useful in cancer or cancer related diseases, disorders. As used herein, "signal-sensor polynucleotides" are nucleic acid transcripts which encode one or more oncology-related polypeptides of interest that, when translated, delivers a "signal" to the cell (cancer or noncancerous) which results in the therapeutic benefit to the organism of either being detrimental to the cancer cell or beneficial to normal cells or

both detrimental to cancer cells and advantageous to normal cells. The signal-sensor polynucleotides may optionally further comprise a sequence (translatable or not) which "senses" the microenvironment of the polynucleotide and alters (a) the function or phenotypic outcome associated with the peptide or protein which is translated, (b) the expression level of the signal-sensor polynucleotide, and/or both.

[0077] The term "nucleic acid," in its broadest sense, includes any compound and/or substance that comprise a polymer of nucleotides. These polymers are often referred to as polynucleotides. Exemplary nucleic acids or polynucleotides of the invention include, but are not limited to, ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs, including LNA having a β- D-ribo configuration, a-LNA having an a-L-ribo configuration (a diastereomer of LNA), 2'-amino-LNA having a 2'-amino functionalization, and 2'-amino- a-LNA having a 2'-amino functionalization) or hybrids thereof.

[0078] In preferred embodiments, the signal-sensor polynucleotide or nucleic acid molecule is a messenger RNA (mRNA). As used herein, the term "messenger RNA" (mRNA) refers to any polynucleotide which encodes a polypeptide of interest and which is capable of being translated to produce the encoded polypeptide of interest *in vitro, in vivo, in situ* or *ex vivo.* Signal-sensor polynucleotides of the invention may be mRNA or any nucleic acid molecule and may or may not be chemically modified.

[0079] Traditionally, the basic components of an mRNA molecule include at least a coding region, a 5'UTR, a 3'UTR, a 5' cap and a poly-A tail. Building on this wild type modular structure, the present invention expands the scope of functionality of traditional mRNA molecules by providing signal-sensor polynucleotides or primary RNA constructs which maintain a modular organization, but which comprise one or more structural and/or chemical modifications or alterations which impart useful properties to the polynucleotide including, in some embodiments, the lack of a substantial induction of the innate immune response of a cell into which the signal-sensor polynucleotide is introduced. As such, modified mRNA molecules of the present invention, which may be synthetic, are termed "mmRNA." As used herein, a "structural" feature or modification is one in which two or more linked nucleotides are inserted, deleted, duplicated, inverted or

randomized in a signal-sensor polynucleotide polynucleotide, primary construct or mmRNA without significant chemical modification to the nucleotides themselves. Because chemical bonds will necessarily be broken and reformed to effect a structural modification, structural modifications are of a chemical nature and hence are chemical modifications. However, structural modifications will result in a different sequence of nucleotides. For example, the polynucleotide "ATCG" may be chemically modified to "AT-5meC-G". The same polynucleotide may be structurally modified from "ATCG" to "ATCCCG". Here, the dinucleotide "CC" has been inserted, resulting in a structural modification to the polynucleotide.

Signal-sensor polynucleotide, primary construct or mmRNA Architecture

[0080] The signal-sensor polynucleotides of the present invention are distinguished from wild type mRNA in their functional and/or structural design features which serve to, as evidenced herein, overcome existing problems of effective polypeptide production using nucleic acid-based therapeutics.

[0081] Figure 1 shows a representative signal-sensor primary construct **100** of the present invention. As used herein, the term "primary construct" or "primary mRNA construct" refers to a signal-sensor polynucleotide transcript which encodes one or more polypeptides of interest and which retains sufficient structural and/or chemical features to allow the polypeptide of interest encoded therein to be translated. Signal-sensor primary constructs may be polynucleotides of the invention. When structurally or chemically modified, the signal-sensor primary construct may be referred to as a mmRNA.

[0082] Returning to FIG. **1,** the primary construct **100** here contains a first region of linked nucleotides **102** that is flanked by a first flanking region **104** and a second flaking region **106.** As used herein, the "first region" may be referred to as a "coding region" or "region encoding" or simply the "first region." This first region may include, but is not limited to, the encoded oncology-related polypeptide of interest. The oncology-related polypeptide of interest may comprise at its 5' terminus one or more signal peptide sequences encoded by a signal peptide sequence region **103.** The flanking region **104** may comprise a region of linked nucleotides comprising one or more complete or incomplete 5' UTRs sequences. The flanking region **104** may also comprise a 5' terminal cap **108.** The second flanking region **106** may comprise a region of linked nucleotides

comprising one or more complete or incomplete 3' UTRs. The flanking region **106** may also comprise a 3' tailing sequence **110** and a 3'UTR **120.**

[0083] Bridging the 5' terminus of the first region **102** and the first flanking region **104** is a first operational region **105.** Traditionally this operational region comprises a start codon. The operational region may alternatively comprise any translation initiation sequence or signal including a start codon.

[0084] Bridging the 3' terminus of the first region **102** and the second flanking region **106** is a second operational region **107.** Traditionally this operational region comprises a stop codon. The operational region may alternatively comprise any translation initiation sequence or signal including a stop codon. According to the present invention, multiple serial stop codons may also be used. In one embodiment, the operation region of the present invention may comprise two stop codons. The first stop codon may be "TGA" and the second stop codon may be selected from the group consisting of "TAA," "TGA" and "TAG." The operation region may further comprise three stop codons. The third stop codon may be selected from the group consisting of "TAA," "TGA" and "TAG."

[0085] Turning to Figure **2,** the 3'UTR **120** of the second flanking region **106** may comprise one or more sensor sequences **130.** A region comprising at least one sensor sequence is referred to as a "sensor region." These sensor sequences as discussed herein operate as pseudo-receptors (or binding sites) for ligands of the local microenvironment of the primary construct or signal-sensor polynucleotide. For example, microRNA bindng sites or miRNA seeds may be used as sensors such that they function as pseudoreceptors for any microRNAs present in the environment of the polynucleotide.

[0086] Generally, the shortest length of the first region of the signal-sensor primary construct of the present invention can be the length of a nucleic acid sequence that is sufficient to encode for a dipeptide, a tripeptide, a tetrapeptide, a pentapeptide, a hexapeptide, a heptapeptide, an octapeptide, a nonapeptide, or a decapeptide. In another embodiment, the length may be sufficient to encode a peptide of 2-30 amino acids, e.g. 5-30, 10-30, 2-25, 5-25, 10-25, or 10-20 amino acids. The length may be sufficient to encode for a peptide of at least 11, 12, 13, 14, 15, 17, 20, 25 or 30 amino acids, or a peptide that is no longer than 40 amino acids, e.g. no longer than 35, 30, 25, 20, 17, 15,

14, 13, 12, 11 or 10 amino acids. Examples of dipeptides that the polynucleotide sequences can encode or include, but are not limited to, carnosine and anserine.

[0087] Generally, the length of the first region encoding the oncology-related polypeptide of interest of the present invention is greater than about 30 nucleotides in length (e.g., at least or greater than about 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,500, and 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000 or up to and including 100,000 nucleotides). As used herein, the "first region" may be referred to as a "coding region" or "region encoding" or simply the "first region."

[0088] In some embodiments, the signal-sensor polynucleotide polynucleotide, primary construct, or mmRNA includes from about 30 to about 100,000 nucleotides (e.g., from 30 to 50, from 30 to 100, from 30 to 250, from 30 to 500, from 30 to 1,000, from 30 to 1,500, from 30 to 3,000, from 30 to 5,000, from 30 to 7,000, from 30 to 10,000, from 30 to 25,000, from 30 to 50,000, from 30 to 70,000, from 100 to 250, from 100 to 500, from 100 to 1,000, from 100 to 1,500, from 100 to 3,000, from 100 to 5,000, from 100 to 7,000, from 100 to 10,000, from 100 to 25,000, from 100 to 50,000, from 100 to 70,000, from 100 to 100,000, from 500 to 1,000, from 500 to 1,500, from 500 to 2,000, from 500 to 3,000, from 500 to 5,000, from 500 to 7,000, from 500 to 10,000, from 500 to 25,000, from 500 to 50,000, from 500 to 70,000, from 500 to 100,000, from 1,000 to 1,500, from 1,000 to 2,000, from 1,000 to 3,000, from 1,000 to 5,000, from 1,000 to 7,000, from 1,000 to 10,000, from 1,000 to 25,000, from 1,000 to 50,000, from 1,000 to 70,000, from 1,000 to 100,000, from 1,500 to 3,000, from 1,500 to 5,000, from 1,500 to 7,000, from 1,500 to 10,000, from 1,500 to 25,000, from 1,500 to 50,000, from 1,500 to 70,000, from 1,500 to 100,000, from 2,000 to 3,000, from 2,000 to 5,000, from 2,000 to 7,000, from 2,000 to 10,000, from 2,000 to 25,000, from 2,000 to 50,000, from 2,000 to 70,000, and from 2,000 to 100,000).

[0089] According to the present invention, the first and second flanking regions may range independently from 15-1,000 nucleotides in length (e.g., greater than 30, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600,

700, 800, and 900 nucleotides or at least 30, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, and 1,000 nucleotides).

[0090] According to the present invention, the tailing sequence may range from absent to 500 nucleotides in length (e.g., at least 60, 70, 80, 90, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, or 500 nucleotides). Where the tailing region is a polyA tail, the length may be determined in units of or as a function of polyA binding protein binding. In this embodiment, the polyA tail is long enough to bind at least 4 monomers of polyA binding protein. PolyA binding protein monomers bind to stretches of approximately 38 nucleotides. As such, it has been observed that polyA tails of about 80 nucleotides and 160 nucleotides are functional.

[0091] According to the present invention, the capping region may comprise a single cap or a series of nucleotides forming the cap. In this embodiment the capping region may be from 1 to 10, e.g. 2-9, 3-8, 4-7, 1-5, 5-10, or at least 2, or 10 or fewer nucleotides in length. In some embodiments, the cap is absent.

[0092] According to the present invention, the first and second operational regions may range from 3 to 40, e.g., 5-30, 10-20, 15, or at least 4, or 30 or fewer nucleotides in length and may comprise, in addition to a start and/or stop codon, one or more signal and/or restriction sequences.

Cyclic signal-sensor polynucleotides

[0093] According to the present invention, a signal-sensor primary construct or mmRNA may be cyclized, or concatemerized, to generate a translation competent molecule to assist interactions between poly-A binding proteins and 5'-end binding proteins. The mechanism of cyclization or concatemerization may occur through at least 3 different routes: 1) chemical, 2) enzymatic, and 3) ribozyme catalyzed. The newly formed 5'-/3'-linkage may be intramolecular or intermolecular.

[0094] In the first route, the 5'-end and the 3'-end of the nucleic acid may contain chemically reactive groups that, when close together, form a new covalent linkage between the 5'-end and the 3'-end of the molecule. The 5'-end may contain an NHS-ester reactive group and the 3'-end may contain a 3'-amino-terminated nucleotide such that in an organic solvent the 3'-amino-terminated nucleotide on the 3'-end of a synthetic mRNA

molecule will undergo a nucleophilic attack on the 5'-NHS-ester moiety forming a new 5'-/3'-amide bond.

[0095] In the second route, T4 RNA ligase may be used to enzymatically link a 5'-phosphorylated nucleic acid molecule to the 3'-hydroxyl group of a nucleic acid forming a new phosphorodiester linkage. In an example reaction, 1μg of a nucleic acid molecule is incubated at 37°C for 1 hour with 1-10 units of T4 RNA ligase (New England Biolabs, Ipswich, MA) according to the manufacturer's protocol. The ligation reaction may occur in the presence of a split oligonucleotide capable of base-pairing with both the 5'- and 3'-region in juxtaposition to assist the enzymatic ligation reaction.

[0096] In the third route, either the 5'-or 3'-end of the cDNA template encodes a ligase ribozyme sequence such that during *in vitro* transcription, the resultant nucleic acid molecule can contain an active ribozyme sequence capable of ligating the 5'-end of a nucleic acid molecule to the 3'-end of a nucleic acid molecule. The ligase ribozyme may be derived from the Group I Intron, Group I Intron, Hepatitis Delta Virus, Hairpin ribozyme or may be selected by SELEX (systematic evolution of ligands by exponential enrichment). The ribozyme ligase reaction may take 1 to 24 hours at temperatures between 0 and 37°C.

Signal-Sensor Polynucleotide Multimers

[0097] According to the present invention, multiple distinct signal-sensor polynucleotides, primary constructs or mmRNA may be linked together through the 3'-end using nucleotides which are modified at the 3'-terminus. Chemical conjugation may be used to control the stoichiometry of delivery into cells. For example, the glyoxylate cycle enzymes, isocitrate lyase and malate synthase, may be supplied into HepG2 cells at a 1:1 ratio to alter cellular fatty acid metabolism. This ratio may be controlled by chemically linking signal-sensor polynucleotides, primary constructs or mmRNA using a 3'-azido terminated nucleotide on one signal-sensor polynucleotide, primary construct or mmRNA species and a C5-ethynyl or alkynyl-containing nucleotide on the opposite signal-sensor polynucleotide, primary construct or mmRNA species. The modified nucleotide is added post-transcriptionally using terminal transferase (New England Biolabs, Ipswich, MA) according to the manufacturer's protocol. After the addition of the 3'-modified nucleotide, the two signal-sensor polynucleotide, primary construct or

mmRNA species may be combined in an aqueous solution, in the presence or absence of copper, to form a new covalent linkage via a click chemistry mechanism as described in the literature.

[0098] In another example, more than two signal-sensor polynucleotides may be linked together using a functionalized linker molecule. For example, a functionalized saccharide molecule may be chemically modified to contain multiple chemical reactive groups (SH-, NH_2-, N_3, etc. ..) to react with the cognate moiety on a 3'-functionalized signal-sensorpolynucleotide molecule (i.e., a 3'-maleimide ester, 3'-NHS-ester, alkynyl). The number of reactive groups on the modified saccharide can be controlled in a stoichiometric fashion to directly control the stoichiometric ratio of conjugated signal-sensor polynucleotide, primary construct or mmRNA.

Signal-sensor polynucleotide Conjugates and Combinations

[0099] In order to further enhance oncology-related protein production, signal-sensor polynucleotide primary constructs or mmRNA of the present invention can be designed to be conjugated to other polynucleotides, oncology-related polypeptides, 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), 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, 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 cancer cell, endothelial cell, or bone cell, hormones and hormone receptors, non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, or a drug.

[00100] Conjugation may result in increased stability and/or half life and may be particularly useful in targeting the signal-sensor polynucleotides, primary constructs or mmRNA to specific sites in the cell, tissue or organism.

[00101] According to the present invention, the signal-sensor polynucleotide mmRNA or primary constructs may be administered with, or further encode one or more of RNAi agents, siRNAs, shRNAs, miRNAs, miRNA binding sites, antisense RNAs, ribozymes,

catalytic DNA, tRNA, RNAs that induce triple helix formation, aptamers or vectors, and the like.

[00102] In one embodiment, the signal-sensor polynucleotides described herein may be conjugated with a moiety to target various cancer cells such as, but not limited to, the moieties described in US Patent Application No. US20 1302 16561, the contents of which are herein incorporated by reference in its entirety. The linkage between the signal-sensor polynucleotides and the cancer targeting moiety may be an acid cleavable linkage that can increase the efficacy of the conjugate such as, but not limited to, the linkages described in US Patent Application No. US20 1302 16561, the contents of which are herein incorporated by reference in its entirety.

Bifunctional signal-sensor polynucleotide

[00103] In one embodiment of the invention are bifunctional signal-sensor polynucleotides (e.g., bifunctional primary constructs or bifunctional mmRNA). As the name implies, bifunctional signal-sensor polynucleotides are those having or capable of at least two functions. These molecules may also by convention be referred to as multi-functional.

[00104] The multiple functionalities of bifunctional signal-sensor polynucleotides may be encoded by the RNA (the function may not manifest until the encoded product is translated) or may be a property of the polynucleotide itself. It may be structural or chemical. Bifunctional modified signal-sensor polynucleotides may comprise a function that is covalently or electrostatically associated with the polynucleotides. Further, the two functions may be provided in the context of a complex of a signal-sensor polynucleotide and another molecule.

[00105] Bifunctional signal-sensor polynucleotides may encode oncology-related peptides which are anti-proliferative. These peptides may be linear, cyclic, constrained or random coil. They may function as aptamers, signaling molecules, ligands or mimics or mimetics thereof. Anti-proliferative peptides may, as translated, be from 3 to 50 amino acids in length. They may be 5-40, 10-30, or approximately 15 amino acids long. They may be single chain, multichain or branched and may form complexes, aggregates or any multi-unit structure once translated.

Noncoding Signal-Sensor Polynucleotides

[00106] As described herein, provided are signal-sensor polynucleotides and primary constructs having sequences that are partially or substantially not translatable, e.g., having a noncoding region. Such noncoding region may be the "first region" of the signal-sensor primary construct. Alternatively, the noncoding region may be a region other than the first region. Such molecules are generally not translated, but can exert an effect on protein production by one or more of binding to and sequestering one or more translational machinery components such as a ribosomal protein or a transfer RNA (tRNA), thereby effectively reducing protein expression in the cell or modulating one or more pathways or cascades in a cell which in turn alters protein levels. The signal-sensor polynucleotide and/or primary construct may contain or encode one or more long noncoding RNA (IncRNA, or lincRNA) or portion thereof, a small nucleolar RNA (sno-RNA), micro RNA (miRNA), small interfering RNA (siRNA) or Piwi-interacting RNA (piRNA).

Auxotrophic Signal-Sensor Polynucleotides

[00107] In one embodiment, the signal-sensor polynucleotides of the present invention may be auxotrophic. As used herein, the term "auxotrophic" refers to signal-sensor polynucleotides that comprise at least one feature that triggers, facilitates or induces the degradation or inactivation of the itself in response to spatial or temporal cues such that oncology-related protein expression is substantially prevented or reduced. Such spatial or temporal cues include the location of the signal-sensor polynucleotide to be translated such as a particular tissue or organ or cellular environment. Also contemplated are cues involving temperature, pH, ionic strength, moisture content, and the like.

[00108] In one embodiment, the feature is located in a terminal region of the signal-sensor polynucleotides of the present invention. As a non-limiting example, the auxotrophic mRNA may contain a miR binding site in the terminal region which binds to a miR expressed in a selected tissue so that the expression of the auxotrophic mRNA is substantially prevented or reduced in the selected tissue. To this end and for example, an auxotrophic mRNA containing a miR-122 binding site will not produce protein if localized to the liver since miR-122 is expressed in the liver and binding of the miR would effectuate destruction of the auxotrophic mRNA. As a non-limiting example, HEK293 cells do not express miR-122 so there would be little to no downregulation of a

signal-sensor polynucleotide having a miR-122 sequence in HEK293 but for hepatocytes which do expression miR-122 there would be a downregulation of a signal-sensor polynucleotide having a miR-122 sequence in hepatocytes (see e.g., the study outlined Example 19). As another non-limiting example, the miR-122 level can be measured in HeLa cells, primary human hepatocytes and primary rat hepatocytes prior to administration with a signal-sensor polynucleotide encoding having at least one miR-122 binding site, miR-122 binding site without the seed sequence or a miR-122 binding site After administration the expression of the signal-sensor polynucleotide can be measured to determine the dampening effect of the miR-122 in the signal-sensor polynucleotide (see e.g., the studies outlined in Examples 41, 42, 43 57, 58 and 59). As yet another non-limiting example, the effectiveness of the miR-122 binding site, miR-122 seed or the miR-122 binding site without the seed in different 3'UTRs may be evaluated in order to determine the proper UTR for the desired outcome such as, but not limited to, the highest dampening effect (see e.g., the study outlined in Example 46).

[00109] In one embodiment, the degradation or inactivation of auxotrophic mRNA may comprise a feature responsive to a change in pH. As a non-limiting example, the auxotrophic mRNA may be triggered in an environment having a pH of between pH 4.5 to 8.0 such as at a pH of 5.0 to 6.0 or a pH of 6.0 to 6.5. The change in pH may be a change of 0.1 unit, 0.2 units, 0.3 units, 0.4 units, 0.5 units, 0.6 units, 0.7 units, 0.8 units, 0.9 units, 1.0 units, 1.1 units, 1.2 units, 1.3 units, 1.4 units, 1.5 units, 1.6 units, 1.7 units, 1.8 units, 1.9 units, 2.0 units, 2.1 units, 2.2 units, 2.3 units, 2.4 units, 2.5 units, 2.6 units, 2.7 units, 2.8 units, 2.9 units, 3.0 units, 3.1 units, 3.2 units, 3.3 units, 3.4 units, 3.5 units, 3.6 units, 3.7 units, 3.8 units, 3.9 units, 4.0 units or more.

[00110] In another embodiment, the degradation or inactivation of auxotrophic mRNA may be triggered or induced by changes in temperature. As a non-limiting example, a change of temperature from room temperature to body temperature. The change of temperature may be less than 1°C, less than 5°C, less than 10°C, less than 15°C, less than 20°C, less than 25°C or more than 25°C.

[00111] In yet another embodiment, the degradation or inactivation of auxotrophic mRNA may be triggered or induced by a change in the levels of ions in the subject. The

ions may be cations or anions such as, but not limited to, sodium ions, potassium ions, chloride ions, calcium ions, magnesium ions and/or phosphate ions.

[00112]

Oncology-related polypeptides of interest

[00113] According to the present invention, the signal-sensor primary construct is designed to encode one or more oncology-related polypeptides of interest or fragments thereof. An oncology-related polypeptide of interest may include, but is not limited to, whole polypeptides, a plurality of polypeptides or fragments of polypeptides, which independently may be encoded by one or more nucleic acids, a plurality of nucleic acids, fragments of nucleic acids or variants of any of the aforementioned. As used herein, the term "oncology-related polypeptides of interest" refers to any polypeptide which is selected to be encoded in the signal-sensor primary construct of the present invention. As used herein, "polypeptide" means a polymer of amino acid residues (natural or unnatural) linked together most often by peptide bonds. The term, as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. In some instances the polypeptide encoded is smaller than about 50 amino acids and the polypeptide is then termed a peptide. If the polypeptide is a peptide, it will be at least about 2, 3, 4, or at least 5 amino acid residues long. Thus, polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing. A polypeptide may be a single molecule or may be a multi-molecular complex such as a dimer, trimer or tetramer. They may also comprise single chain or multichain polypeptides such as antibodies or insulin and may be associated or linked. Most commonly disulfide linkages are found in multichain polypeptides. The term polypeptide may also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analogue of a corresponding naturally occurring amino acid.

[00114] The term "polypeptide variant" refers to molecules which differ in their amino acid sequence from a native or reference sequence. The amino acid sequence variants may possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence, as compared to a native or reference sequence. Ordinarily, variants will possess at least about 50% identity (homology) to a native or reference sequence,

and preferably, they will be at least about 80%, more preferably at least about 90% identical (homologous) to a native or reference sequence.

[00115] In some embodiments "variant mimics" are provided. As used herein, the term "variant mimic" is one which contains one or more amino acids which would mimic an activated sequence. For example, glutamate may serve as a mimic for phosphoro-threonine and/or phosphoro-serine. Alternatively, variant mimics may result in deactivation or in an inactivated product containing the mimic, e.g., phenylalanine may act as an inactivating substitution for tyrosine; or alanine may act as an inactivating substitution for serine.

[00116] "Homology" as it applies to amino acid sequences is defined as the percentage of residues in the candidate amino acid sequence that are identical with the residues in the amino acid sequence of a second sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology. Methods and computer programs for the alignment are well known in the art. It is understood that homology depends on a calculation of percent identity but may differ in value due to gaps and penalties introduced in the calculation.

[00117] By "homologs" as it applies to polypeptide sequences means the corresponding sequence of other species having substantial identity to a second sequence of a second species.

[00118] "Analogs" is meant to include polypeptide variants which differ by one or more amino acid alterations, e.g., substitutions, additions or deletions of amino acid residues that still maintain one or more of the properties of the parent or starting polypeptide.

[00119] The present invention contemplates several types of compositions which are polypeptide based including variants and derivatives. These include substitutional, insertional, deletion and covalent variants and derivatives. The term "derivative" is used synonymously with the term "variant" but generally refers to a molecule that has been modified and/or changed in any way relative to a reference molecule or starting molecule.

[00120] As such, signal-sensor polynucleotides encoding oncology-related polypeptides containing substitutions, insertions and/or additions, deletions and covalent modifications with respect to reference sequences, in particular the oncology-related polypeptide sequences disclosed herein, are included within the scope of this invention. For example,

sequence tags or amino acids, such as one or more lysines, can be added to the peptide sequences of the invention (e.g., at the N-terminal or C-terminal ends). Sequence tags can be used for peptide purification or localization. Lysines can be used to increase peptide solubility or to allow for biotinylation. Alternatively, amino acid residues located at the carboxy and amino terminal regions of the amino acid sequence of a peptide or protein may optionally be deleted providing for truncated sequences. Certain amino acids (e.g., C-terminal or N-terminal residues) may alternatively be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support.

[00121] "Substitutional variants" when referring to polypeptides are those that have at least one amino acid residue in a native or starting sequence removed and a different amino acid inserted in its place at the same position. The substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule.

[00122] As used herein the term "conservative amino acid substitution" refers to the substitution of an amino acid that is normally present in the sequence with a different amino acid of similar size, charge, or polarity. Examples of conservative substitutions include the substitution of a non-polar (hydrophobic) residue such as isoleucine, valine and leucine for another non-polar residue. Likewise, examples of conservative substitutions include the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, and between glycine and serine. Additionally, the substitution of a basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue such as aspartic acid or glutamic acid for another acidic residue are additional examples of conservative substitutions. Examples of non-conservative substitutions include the substitution of a non-polar (hydrophobic) amino acid residue such as isoleucine, valine, leucine, alanine, methionine for a polar (hydrophilic) residue such as cysteine, glutamine, glutamic acid or lysine and/or a polar residue for a non-polar residue.

[00123] "Insertional variants" when referring to polypeptides are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in a

native or starting sequence. "Immediately adjacent" to an amino acid means connected to either the alpha-carboxy or alpha-amino functional group of the amino acid.

[00124] "Deletional variants" when referring to polypeptides are those with one or more amino acids in the native or starting amino acid sequence removed. Ordinarily, deletional variants will have one or more amino acids deleted in a particular region of the molecule.

[00125] "Covalent derivatives" when referring to polypeptides include modifications of a native or starting protein with an organic proteinaceous or non-proteinaceous derivatizing agent, and/or post-translational modifications. Covalent modifications are traditionally introduced by reacting targeted amino acid residues of the protein with an organic derivatizing agent that is capable of reacting with selected side-chains or terminal residues, or by harnessing mechanisms of post-translational modifications that function in selected recombinant host cells. The resultant covalent derivatives are useful in programs directed at identifying residues important for biological activity, for immunoassays, or for the preparation of anti-protein antibodies for immunoaffinity purification of the recombinant glycoprotein. Such modifications are within the ordinary skill in the art and are performed without undue experimentation.

[00126] Certain post-translational modifications are the result of the action of recombinant host cells on the expressed oncology-related polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues may be present in the oncology-related polypeptides produced in accordance with the present invention.

[00127] Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)).

[00128] "Features" when referring to polypeptides are defined as distinct amino acid sequence-based components of a molecule. Features of the polypeptides encoded by the mmRNA of the present invention include surface manifestations, local conformational

shape, folds, loops, half-loops, domains, half-domains, sites, termini or any combination thereof.

[00129] As used herein when referring to polypeptides the term "surface manifestation" refers to a polypeptide based component of a protein appearing on an outermost surface.

[00130] As used herein when referring to polypeptides the term "local conformational shape" means a polypeptide based structural manifestation of a protein which is located within a definable space of the protein.

[00131] As used herein when referring to polypeptides the term "fold" refers to the resultant conformation of an amino acid sequence upon energy minimization. A fold may occur at the secondary or tertiary level of the folding process. Examples of secondary level folds include beta sheets and alpha helices. Examples of tertiary folds include domains and regions formed due to aggregation or separation of energetic forces. Regions formed in this way include hydrophobic and hydrophilic pockets, and the like.

[00132] As used herein the term "turn" as it relates to protein conformation means a bend which alters the direction of the backbone of a peptide or polypeptide and may involve one, two, three or more amino acid residues.

[00133] As used herein when referring to polypeptides the term "loop" refers to a structural feature of a polypeptide which may serve to reverse the direction of the backbone of a peptide or polypeptide. Where the loop is found in a polypeptide and only alters the direction of the backbone, it may comprise four or more amino acid residues. Oliva et al. have identified at least 5 classes of protein loops (J. Mol Biol 266 (4): 814-830; 1997). Loops may be open or closed. Closed loops or "cyclic" loops may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids between the bridging moieties. Such bridging moieties may comprise a cysteine-cysteine bridge (Cys-Cys) typical in polypeptides having disulfide bridges or alternatively bridging moieties may be non-protein based such as the dibromozylyl agents used herein.

[00134] As used herein when referring to polypeptides the term "half-loop" refers to a portion of an identified loop having at least half the number of amino acid resides as the loop from which it is derived. It is understood that loops may not always contain an even number of amino acid residues. Therefore, in those cases where a loop contains or is identified to comprise an odd number of amino acids, a half-loop of the odd-numbered

loop will comprise the whole number portion or next whole number portion of the loop (number of amino acids of the loop/2+/-0.5 amino acids). For example, a loop identified as a 7 amino acid loop could produce half-loops of 3 amino acids or 4 amino acids (7/2=3.5+/-0.5 being 3 or 4).

[00135] As used herein when referring to polypeptides the term "domain" refers to a motif of a polypeptide having one or more identifiable structural or functional characteristics or properties (e.g., binding capacity, serving as a site for protein-protein interactions).

[00136] As used herein when referring to polypeptides the term "half-domain" means a portion of an identified domain having at least half the number of amino acid resides as the domain from which it is derived. It is understood that domains may not always contain an even number of amino acid residues. Therefore, in those cases where a domain contains or is identified to comprise an odd number of amino acids, a half-domain of the odd-numbered domain will comprise the whole number portion or next whole number portion of the domain (number of amino acids of the domain/2+/-0.5 amino acids). For example, a domain identified as a 7 amino acid domain could produce half-domains of 3 amino acids or 4 amino acids (7/2=3.5+/-0.5 being 3 or 4). It is also understood that sub-domains may be identified within domains or half-domains, these subdomains possessing less than all of the structural or functional properties identified in the domains or half domains from which they were derived. It is also understood that the amino acids that comprise any of the domain types herein need not be contiguous along the backbone of the polypeptide (i.e., nonadjacent amino acids may fold structurally to produce a domain, half-domain or subdomain).

[00137] As used herein when referring to polypeptides the terms "site" as it pertains to amino acid based embodiments is used synonymously with "amino acid residue" and "amino acid side chain." A site represents a position within a peptide or polypeptide that may be modified, manipulated, altered, derivatized or varied within the polypeptide based molecules of the present invention.

[00138] As used herein the terms "termini" or "terminus" when referring to polypeptides refers to an extremity of a peptide or polypeptide. Such extremity is not limited only to the first or final site of the peptide or polypeptide but may include additional amino acids

in the terminal regions. The polypeptide based molecules of the present invention may be characterized as having both an N-terminus (terminated by an amino acid with a free amino group (NH2)) and a C-terminus (terminated by an amino acid with a free carboxyl group (COOH)). Proteins of the invention are in some cases made up of multiple polypeptide chains brought together by disulfide bonds or by non-covalent forces (multimers, oligomers). These sorts of proteins will have multiple N- and C-termini. Alternatively, the termini of the polypeptides may be modified such that they begin or end, as the case may be, with a non-polypeptide based moiety such as an organic conjugate.

[00139] Once any of the features have been identified or defined as a desired component of a polypeptide to be encoded by the signal-sensor primary construct or mmRNA of the invention, any of several manipulations and/or modifications of these features may be performed by moving, swapping, inverting, deleting, randomizing or duplicating. Furthermore, it is understood that manipulation of features may result in the same outcome as a modification to the molecules of the invention. For example, a manipulation which involved deleting a domain would result in the alteration of the length of a molecule just as modification of a nucleic acid to encode less than a full length molecule would.

[00140] Modifications and manipulations can be accomplished by methods known in the art such as, but not limited to, site directed mutagenesis. The resulting modified molecules may then be tested for activity using *in vitro* or *in vivo* assays such as those described herein or any other suitable screening assay known in the art.

[00141] According to the present invention, the oncology-related polypeptides may comprise a consensus sequence which is discovered through rounds of experimentation. As used herein a "consensus" sequence is a single sequence which represents a collective population of sequences allowing for variability at one or more sites.

[00142] As recognized by those skilled in the art, protein fragments, functional protein domains, and homologous proteins are also considered to be within the scope of oncology-related polypeptides of interest of this invention. For example, provided herein is any protein fragment (meaning an oncology-related polypeptide sequence at least one amino acid residue shorter than a reference oncology-related polypeptide sequence but

otherwise identical) of a reference oncology-related protein 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or greater than 100 amino acids in length. In another example, any oncology-related protein that includes a stretch of about 20, about 30, about 40, about 50, or about 100 amino acids which are about 40%, about 50%, about 60%>, about 70%>, about 80%>, about 90%, about 95%, or about *100%* identical to any of the sequences described herein can be utilized in accordance with the invention. In certain embodiments, a polypeptide to be utilized in accordance with the invention includes 2, 3, 4, 5, 6, 7, 8, 9, 10, or more mutations as shown in any of the sequences provided or referenced herein.

Encoded Oncology-Related Polypeptides

[00143] The signal-sensor primary constructs or mmRNA of the present invention may be designed to encode oncology-related polypeptides of interest such as oncology-related peptides and proteins.

[00144] In one embodiment, signal-sensor primary constructs or mmRNA of the present invention may encode variant polypeptides which have a certain identity with a reference oncology-related polypeptide sequence. As used herein, a "reference oncology-related polypeptide sequence" refers to a starting oncology-related polypeptide sequence. Reference sequences may be wild type sequences or any sequence to which reference is made in the design of another sequence. A "reference polypeptide sequence" may, e.g., be any one of the protein sequence listed in Table 6.

[00145] The term "identity" as known in the art, refers to a relationship between the sequences of two or more peptides, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between peptides, as determined by the number of matches between strings of two or more amino acid residues. Identity measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., "algorithms"). Identity of related peptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular

Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al, SIAM J. Applied Math. 48, 1073 (1988).

[00146] In some embodiments, the polypeptide variant may have the same or a similar activity as the reference oncology-related polypeptide. Alternatively, the variant may have an altered activity (e.g., increased or decreased) relative to a reference oncology-related polypeptide. Generally, variants of a particular signal-sensor polynucleotide or oncology-related polypeptide of the invention will have at least about 40%, 45%>, 50%>, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, **98%o,** 99%, but less than 100% sequence identity to that particular reference signal-sensor polynucleotide or oncology-related polypeptide as determined by sequence alignment programs and parameters described herein and known to those skilled in the art. Such tools for alignment include those of the BLAST suite (Stephen F. Altschul, Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.) Other tools are described herein, specifically in the definition of "identity."

[00147] Default parameters in the BLAST algorithm include, for example, an expect threshold of 10, Word size of 28, Match/Mismatch Scores 1, -2, Gap costs Linear. Any filter can be applied as well as a selection for species specific repeats, e.g., Homo sapiens.

[00148] In one embodiment, the signal-sensor polynucleotides, primary constructs and/or mmRNA may be used to treat a disease, disorder and/or condition in a subject.

[00149] In one embodiment, the polynucleotides, primary constructs and/or mmRNA may be used to reduce, eliminate or prevent tumor growth in a subject.

[00150] In one embodiment, the signal-sensor polynucleotides, primary constructs and/or mmRNA may be used to recude and/or ameliorate at least one symptom of cancer in a subject. A symptom of cancer may include, but is not limited to, weakness, aches and pains, fever, fatigue, weight loss, blood clots, increased blood calcium levels, low white blood cell count, short of breath, dizziness, headaches, hyperpigmentation, jaundice, erthema, pruritis, excessive hair growth, change in bowel habits, change in bladder function, long-lasting sores, white patches inside the mouth, white spots on the

tongue, unusual bleeding or discharge, thickening or lump on parts of the body, indigestion, trouble swallowing, changes in warts or moles, change in new skin and nagging cough or hoarseness. Further, the signal-sensor polynucleotides, primary constructs and/or mmRNA may reduce a side-effect associated with cancer such as, but not limited to, chemo brain, peripheral neuropathy, fatigue, depression, nausea, vomiting, pain, anemia, lymphedema, infections, sexual side effects, reduced fertility or infertility, ostomies, insomnia and hair loss.

Oncology-relatedproteins or oncology-relatedpeptides

[00151] The signal-sensor primary constructs or mmRNA disclosed herein, may encode one or more validated or "in testing" oncology-related proteins or oncology-related peptides.

[00152] According to the present invention, one or more oncology-related proteins or oncology-related peptides currently being marketed or in development may be encoded by the oncology-related signal-sensor polynucleotide, primary constructs or mmRNA of the present invention. While not wishing to be bound by theory, it is believed that incorporation into the signal-sensor primary constructs or mmRNA of the invention will result in improved therapeutic efficacy due at least in part to the specificity, purity and selectivity of the construct designs.

[00153] The signal-sensor polynucleotides, primary constructs and/or mmRNA may alter a biological and/or physiolocial process and/or compound such as, but not limited to, the cell cycle, the DNA damage response (e.g., DNA damage repair), apoptosis, angiogenesis, cell motility, the epithelial to mesenchymal transition in epithelial cells, the phosphatidyl inositol 3 (PI3) kinase/Akt cellular signaling pathway, telomerase activity and/or expression, tumor metastasis, tumorigenesis, cathepsins, cell senescence, receptor tyrosine kinase signaling, metabolism and drug metabolism, G protein signaling, growth factors and receptors, heat shock proteins, histone deacetylases, hormone receptors, hypoxia, poly ADP-ribose polymerases, protein kinases, RAS signaling, topisomerases, transcription factors and tumor suppressor activity in cancerous, precancerous and/or other cells.

[00154] In one embodiment, the signal-sensor polynucleotides, primary constructs and/or mmRNA may used to express a polypeptide in cells or tissues for the purpose of replacing the protein produced from a deleted or mutated gene.

[00155] Further, the polynucleotides, primary constructs or mmRNA of the invention may be used to treat cancer which has been caused by carcinogens of natural and/or synthetic origin. In another embodiment, the use of the polynucleotides, primary constructs and/or mmRNA may be used to treat cancer caused by other organisms and/or cancers caused by viral infection.

Sensors in the flanking regions: Untranslated Regions (UTRs)

[00156] Untranslated regions (UTRs) of a gene are transcribed but not translated. The 5'UTR starts at the transcription start site and continues to the start codon but does not include the start codon; whereas, the 3'UTR starts immediately following the stop codon and continues until the transcriptional termination signal. There is growing body of evidence about the regulatory roles played by the UTRs in terms of stability of the nucleic acid molecule and translation. The regulatory features of a UTR can be incorporated into the signal-sensor polynucleotides, primary constructs and/or mmRNA of the present invention to enhance the stability of the molecule. The specific features can also be incorporated to ensure controlled down-regulation of the transcript in case they are misdirected to undesired organs sites. The untranslated regions may be incorporated into a vector system which can produce mRNA and/or be delivered to a cell, tissue and/or organism to produce a polypeptide of interest.

[00157] In one embodiment, the signal-sensor polynucleotides, primary constructs and/or mmRNA of the present may comprise at least one terminal modification. Non-limiting examples of terminal modifications are described in US Provisional Patent Application No US 61/729,933, filed November 26, 2012, entitled Terminally Optimized Modified RNAs, US Provisional Patent application No US 61/737,224, filed December 14, 2012, entitled Terminally Optimized RNAs, US Provisional Patent Application No US 61/758,921, filed January 31, 2013, entitled Differential Targeting Using RNA Constructs, US Provisional Patent Application No. US 61/781,139, filed March 14, 2013, entitled Differential Targeting Using RNA Constructs, US Provisional Patent Application No US 61/829,359, filed May 31, 2013, entitled Differential Targeting Using

RNA Constructs, US Provisional Patent Application No. 61/839,903, filed June 27, 2013, entitled Differential Targeting Using RNA Constructs, US Provisional Patent Application No. 61/842,709, filed July 3, 2013, entitled Differential Targeting Using RNA Constructs, and US Provisional Patent Application No. 61/857,436, filed July 23, 2013, entitled Differential Targeting Using RNA Constructs, the contents of each of which are herein incorporated by reference in their entireties. These terminal modifications include, but are not limited to, 5'caps, microRNA binding sites in the terminal region, chain terminating nucleosides, translation enhancer elements in the terminal region and tailing sequences including a polyA-G quartet and stem loop sequences.

5' UTR and Translation Initiation

[00158] Natural 5'UTRs bear features which play roles in for translation initiation. They harbor signatures like Kozak sequences which are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus CCR(A/G)CCAUGG, where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G'. 5'UTR also have been known to form secondary structures which are involved in elongation factor binding. For example, one of the secondary 5'-UTR structures is the structured IRES for eIF4A2 elongation factor binding, which is necessary for the microRNA mediated gene repression at 3'-UTR.

[00159] 5'UTR secondary structures involved in elongation factor binding can interact with other RNA binding molecules in the 5'UTR or 3'UTR to regulate gene expression. For example, the elongation factor EIF4A2 binding to a secondarily structured element in the 5'UTR is necessary for microRNA mediated repression (Meijer HA et al., Science, 2013, 340, 82-85, herein incorporated by reference in its entirety). The different secondary structures in the 5'UTR can be incorporated into the flanking region to either stabilize or selectively destalized mRNAs in specific tissues or cells.

[00160] By engineering the features typically found in abundantly expressed genes of specific target organs, one can enhance the stability and oncology-related protein production of the signal-sensor polynucleotides, primary constructs or mmRNA of the invention. For example, introduction of 5' UTR of liver-expressed mRNA, such as albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein,

erythropoietin, or Factor VIII, could be used to enhance expression of a nucleic acid molecule, such as a mmRNA, in hepatic cell lines or liver. Likewise, use of 5' UTR from other tissue-specific mRNA to improve expression in that tissue is possible - for muscle (MyoD, Myosin, Myoglobin, Myogenin, Herculin), for endothelial cells (Tie-1, CD36), for myeloid cells (C/EBP, AML1, G-CSF, GM-CSF, CD1 lb, MSR, Fr-1, i-NOS), for leukocytes (CD45, CD18), for adipose tissue (CD36, GLUT4, ACRP30, adiponectin) and for lung epithelial cells (SP-A/B/C/D).

[00161] Other non-UTR sequences may be incorporated into the 5' (or 3' UTR) UTRs. For example, introns or portions of introns sequences may be incorporated into the flanking regions of the signal-sensor polynucleotides, primary constructs or mmRNA of the invention. Incorporation of intronic sequences may increase protein production as well as mRNA levels.

Translation Enhancer Elements (TEEs)

[00162] In one embodiment, the 5'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA may include at least one translational enhancer polynucleotide, translation enhancer element, translational enhancer elements (collectively referred to as "TEE"s). As a non-limiting example, the TEE may be located between the transcription promoter and the start codon. The signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA with at least one TEE in the 5'UTR may include a cap at the 5'UTR. Further, at least one TEE may be located in the 5'UTR of signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA undergoing cap-dependent or cap-independent translation.

[00163] The term "translational enhancer element" or "translation enhancer element" (herein collectively referred to as "TEE") refers to sequences that increase the amount of polypeptide or protein produced from an mRNA.

[00164] In one embodiment, TEEs are conserved elements in the UTR which can promote translational activity of a nucleic acid such as, but not limited to, cap-dependent or cap-independent translation. The conservation of these sequences has been previously shown by Panek et al (Nucleic Acids Research, 2013, 1-10; herein incorporated by reference in its entirety) across 14 species including humans.

[00165] In one embodiment, the TEE may be any of the TEEs listed in Table 35 in Example 45, including portion and/or fragments thereof. The TEE sequence may include at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or more than 99% of the TEE sequences disclosed in Table 35 and/or the TEE sequence may include a 5-30 nucleotide fragment, a 5-25 nucleotide fragment, a 5-20 nucleotide fragment, a 5-15 nucleotide fragment, a 5-10 nucleotide fragment of the TEE sequences disclosed in Table 35.

[00166] In one non-limiting example, the TEEs known may be in the 5'-leader of the Gtx homeodomain protein (Chappell et al, Proc. Natl. Acad. Sci. USA 101:9590-9594, 2004, herein incorporated by reference in their entirety).

[00167] In another non-limiting example, TEEs are disclosed as SEQ ID NOs: 1-35 in US Patent Publication No. US20090226470, SEQ ID NOs: 1-35 in US Patent Publication US20130177581, SEQ ID NOs: 1-35 in International Patent Publication No. WO2009075886, SEQ ID NOs: 1-5, and 7-645 in International Patent Publication No. WO2012009644, SEQ ID NO: 1 in International Patent Publication No. WO1999024595, SEQ ID NO: 1 in US Patent No. US6310197, and SEQ ID NO: 1 in US Patent No. US6849405, each of which is herein incorporated by reference in its entirety.

[00168] In yet another non-limiting example, the TEE may be an internal ribosome entry site (IRES), HCV-IRES or an IRES element such as, but not limited to, those described in US Patent No. US7468275, US Patent Publication Nos. US20070048776 and US20110124100 and International Patent Publication Nos. WO2007025008 and WO2001055369, each of which is herein incorporated by reference in its entirety. The IRES elements may include, but are not limited to, the Gtx sequences (e.g., Gtx9-nt, Gtx8-nt, Gtx7-nt) described by Chappell et al. (Proc. Natl. Acad. Sci. USA 101:9590-9594, 2004) and Zhou et al. (PNAS 102:6273-6278, 2005) and in US Patent Publication Nos. US20070048776 and US20110124100 and International Patent Publication No. WO2007025008, each of which is herein incorporated by reference in its entirety.

[00169] "Translational enhancer polynucleotides" or "translation enhancer polynucleotide sequences" are polynucleotides which include one or more of the specific

TEE exemplified herein and/or disclosed in the art (see e.g., US63 10197, US6849405, US7456273, US7183395, US20090226470, US20070048776, US201 10124100, US20090093049, US20130177581, WO2009075886, WO2007025008, WO2012009644, WO2001055371 WO1999024595, and EP26 10341 A l and EP2610340A1; each of which is herein incorporated by reference in its entirety) or their variants, homologs or functional derivatives. One or multiple copies of a specific TEE can be present in the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA. The TEEs in the translational enhancer polynucleotides can be organized in one or more sequence segments. A sequence segment can harbor one or more of the specific TEEs exemplified herein, with each TEE being present in one or more copies. When multiple sequence segments are present in a translational enhancer polynucleotide, they can be homogenous or heterogeneous. Thus, the multiple sequence segments in a translational enhancer polynucleotide can harbor identical or different types of the specific TEEs exemplified herein, identical or different number of copies of each of the specific TEEs, and/or identical or different organization of the TEEs within each sequence segment.

[00170] In one embodiment, the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA may include at least one TEE that is described in International Patent Publication No. WO1999024595, WO2012009644, WO2009075886, WO2007025008, WO1999024595, European Patent Publication No. EP2610341A1 and EP2610340A1, US Patent No. US6310197, US6849405, US7456273, US7183395, US Patent Publication No. US20090226470, US201 10124100, US20070048776, US20090093049 and US20130177581, each of which is herein incorporated by reference in its entirety. The TEE may be located in the 5'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA.

[00171] In another embodiment, the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA may include at least one TEE that has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identity with the TEEs described in US Patent Publication Nos. US20090226470, US20070048776, US20130177581 and US201 10124100, International Patent Publication No. WO1999024595, WO2012009644,

WO2009075886 and WO2007025008, European Patent Publication No. EP2610341A1 and EP2610340A1, US Patent No. US6310197, US6849405, US7456273, US7183395, each of which is herein incorporated by reference in its entirety.

[00172] In one embodiment, the 5'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA may include at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55 or more than 60 TEE sequences. The TEE sequences in the 5'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may be the same or different TEE sequences. The TEE sequences may be in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times. In these patterns, each letter, A, B, or C represent a different TEE sequence at the nucleotide level.

[00173] In one embodiment, the 5'UTR may include a spacer to separate two TEE sequences. As a non-limiting example, the spacer may be a 15 nucleotide spacer and/or other spacers known in the art. As another non-limiting example, the 5'UTR may include a TEE sequence-spacer module repeated at least once, at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times and at least 9 times or more than 9 times in the 5'UTR.

[00174] In another embodiment, the spacer separating two TEE sequences may include other sequences known in the art which may regulate the translation of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention such as, but not limited to, miR sequences described herein (e.g., miR binding sites and miR seeds). As a non-limiting example, each spacer used to separate two TEE sequences may include a different miR sequence or component of a miR sequence (e.g., miR seed sequence).

[00175] In one embodiment, the TEE in the 5'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may include at least 5%, at least 10%, at least 15%>, at least 20%>, at least 25%>, at least 30%>, at

least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or more than 99% of the TEE sequences disclosed in US Patent Publication Nos. US20090226470, US20070048776, US20130177581 and US201 10124100, International Patent Publication No. WO1999024595, WO2012009644, WO2009075886 and WO2007025008, European Patent Publication No. EP2610341A1 and EP2610340A1, US Patent No. US6310197, US6849405, US7456273, US7183395. In another embodiment, the TEE in the 5'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may include a 5-30 nucleotide fragment, a 5-25 nucleotide fragment, a 5-20 nucleotide fragment, a 5-15 nucleotide fragment, a 5-10 nucleotide fragment of the TEE sequences disclosed in US Patent Publication Nos. US20090226470, US20070048776, US20130177581 and US201 10124100, International Patent Publication No. WO1999024595, WO2012009644, WO2009075886 and WO2007025008, European Patent Publication No. EP2610341A1 and EP2610340A1, US Patent No. US6310197, US6849405, US7456273, US7183395; each of which are herein incorporated by reference in their entirety.

[00176] In one embodiment, the TEE in the 5'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may include at least 5%, at least 10%>, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or more than 99% of the TEE sequences disclosed in Chappell et al. (Proc. Natl. Acad. Sci. USA 101:9590-9594, 2004) and Zhou et al. (PNAS 102:6273-6278, 2005), in Supplemental Table 1 and in Supplemental Table 2 disclosed by Wellensiek et al (Genome-wide profiling of human cap-independent translation-enhancing elements, Nature Methods, 2013; DOL10.1038/NMETH.2522); each of which is herein incorporated by reference in its entirety. In another embodiment, the TEE in the 5'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may include a 5-30 nucleotide fragment, a 5-25 nucleotide fragment, a 5-20 nucleotide fragment, a 5-15 nucleotide fragment, a 5-10 nucleotide fragment of the TEE sequences disclosed in Chappell et al. (Proc. Natl. Acad.

Sci. USA 101:9590-9594, 2004) and Zhou et al. (PNAS 102:6273-6278, 2005), in Supplemental Table 1 and in Supplemental Table 2 disclosed by Wellensiek et al (Genome-wide profiling of human cap-independent translation-enhancing elements, Nature Methods, 2013; DOL10.1038/NMETH.2522); each of which is herein incorporated by reference in its entirety.

[00177] In one embodiment, the TEE used in the 5'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention is an IRES sequence such as, but not limited to, those described in US Patent No. US7468275 and International Patent Publication No. WO200 1055369, each of which is herein incorporated by reference in its entirety.

[00178] In one embodiment, the TEEs used in the 5'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may be identified by the methods described in US Patent Publication No. US20070048776 and US201 10124100 and International Patent Publication Nos. WO2007025008 and WO2012009644, each of which is herein incorporated by reference in its entirety.

[00179] In another embodiment, the TEEs used in the 5'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may be a transcription regulatory element described in US Patent No. US7456273 and US7183395, US Patent Publication No. US20090093049, and International Publication No. WO200 1055371, each of which is herein incorporated by reference in their entirety. The transcription regulatory elements may be identified by methods known in the art, such as, but not limited to, the methods described in US Patent No. US7456273 and US7183395, US Patent Publication No. US20090093049, and International Publication No. WO200 1055371, each of which is herein incorporated by reference in their entirety.

[00180] In yet another embodiment, the TEE used in the 5'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention is an oligonucleotide or portion thereof as described in US Patent No. US7456273 and US7183395, US Patent Publication No. US20090093049, and

International Publication No. WO200 1055371, each of which is herein incorporated by reference in their entirety.

[00181] The 5' UTR comprising at least one TEE described herein may be incorporated in a monocistronic sequence such as, but not limited to, a vector system or a nucleic acid vector. As a non-limiting example, the vector systems and nucleic acid vectors may include those described in US Patent Nos. 7456273 and US7183395, US Patent Publication No. US20070048776, US20090093049 and US201 10124100 and International Patent Publication Nos. WO2007025008 and WO200 1055371, each of which is herein incorporated by reference in its entirety.

[00182] In one embodiment, the TEEs described herein may be located in the 5'UTR and/or the 3'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA. The TEEs located in the 3'UTR may be the same and/or different than the TEEs located in and/or described for incorporation in the 5'UTR.

[00183] In one embodiment, the 3'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA may include at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55 or more than 60 TEE sequences. The TEE sequences in the 3'UTR of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention may be the same or different TEE sequences. The TEE sequences may be in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times. In these patterns, each letter, A, B, or C represent a different TEE sequence at the nucleotide level.

[00184] In one embodiment, the 3'UTR may include a spacer to separate two TEE sequences. As a non-limiting example, the spacer may be a 15 nucleotide spacer and/or other spacers known in the art. As another non-limiting example, the 3'UTR may include a TEE sequence-spacer module repeated at least once, at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times and at least 9 times or more than 9 times in the 3'UTR.

[00185] In another embodiment, the spacer separating two TEE sequences may include other sequences known in the art which may regulate the translation of the signal-sensor polynucleotides, primary constructs, modified nucleic acids and/or mmRNA of the present invention such as, but not limited to, miR sequences described herein (e.g., miR binding sites and miR seeds). As a non-limiting example, each spacer used to separate two TEE sequences may include a different miR sequence or component of a miR sequence (e.g., miR seed sequence).

[00186] In one embodiment, the incorporation of a miR sequence and/or a TEE sequence changes the shape of the stem loop region which may increase and/or descrease translation. (see e.g, Kedde et al. A Pumilio-induced RNA structure switch in p27-3'UTR controls miR-221 and miR-22 accessibility. Nature Cell Biology. 2010, herein incorporated by reference in its entirety).

[00187] In one embodiment, the 5'UTR may comprise at least one microRNA sequence. The microRNA sequence may be, but is not limited to, a 19 or 22 nucleotide sequence and/or a microRNA sequence without the seed.

[00188] In one embodiment the microRNA sequence in the 5'UTR may be used to stabilize the nucleic acid and/or mRNA described herein.

[00189] In another embodiment, a microRNA sequence in the 5'UTR may be used to decrease the accessibility of the site of translation initiation such as, but not limited to a start codon. Matsuda et al (PLoS One. 2010 1l(5):e 15057; herein incorporated by reference in its entirety) used antisense locked nucleic acid (LNA) oligonucleotides and exon-junctino complexes (EJCs) around a start codon (-4 to +37 where the A of the AUG codons is +1) in order to decrease the accessibility to the first start codon (AUG). Matsuda showed that altering the sequence around the start codon with an LNA or EJC the efficiency, length and structural stability of the nucleic acid or mRNA is affected. The signal-sensor polynucleotides of the present invention may comprise a microRNA sequence, instead of the LNA or EJC sequence described by Matsuda et al, near the site of translation initiation in order to decrease the accessibility to the site of translation initiation. The site of translation initiation may be prior to, after or within the microRNA sequence. As a non-limiting example, the site of translation initiation may be located within a microRNA sequence such as a seed sequence or binding site. As another non-

limiting example, the site of translation initiation may be located within a miR-122 sequence such as the seed sequence or the mir-122 binding site.

[00190] In one embodiment, the nucleic acids or mRNA of the present invention comprises at least one microRNA sequence in a region of the nucleic acid or mRNA which may interact with a RNA binding protein.

RNA Motifs for RNA Binding Proteins (RBPs)

[00191] RNA binding proteins (RBPs) can regulate numerous aspects of co- and post-transcription gene expression such as, but not limited to, RNA splicing, localization, translation, turnover, polyadenylation, capping, modification, export and localization. RNA-binding domains (RBDs), such as, but not limited to, RNA recognition motif (RR) and hnRNP K-homology (KH) domains, typically regulate the sequence association between RBPs and their RNA targets (Ray et al. Nature 2013. 499:172-177; herein incorporated by reference in its entirety). In one embodiment, the canonical RBDs can bind short RNA sequences. In another embodiment, the canonical RBDs can recognize structure RNAs.

[00192] In one embodiment, the nucleic acids and/or mRNA may comprise at least one RNA-binding motif such as, but not limited to a RNA-binding domain (RBD).

[00193] In one embodiment, the RBD may be any of the RBDs, fragments or variants thereof descried by Ray et al. (Nature 2013. 499:172-177; herein incorporated by reference in its entirety).

[00194] In one embodiment, the nucleic acids or mRNA of the present invention may comprise a sequence for at least one RNA-binding domain (RBDs). When the nucleic acids or mRNA of the present invention comprise more than one RBD, the RBDs do not need to be from the same species or even the same structural class.

[00195] In one embodiment, at least one flanking region (e.g., the 5'UTR and/or the 3'UTR) may comprise at least one RBD. In another embodiment, the first flanking region and the second flanking region may both comprise at least one RBD. The RBD may be the same or each of the RBDs may have at least 60% sequence identity to the other RBD. As a non-limiting example, at least on RBD may be located before, after and/or within the 3'UTR of the nucleic acid or mRNA of the present invention. As

another non-limiting example, at least one RBD may be located before or within the first 300 nucleosides of the 3'UTR.

[00196] In another embodiment, the nucleic acids and/or mRNA of the present invention may comprise at least one RBD in the first region of linked nucleosides. The RBD may be located before, after or within a coding region (e.g., the ORF).

[00197] In yet another embodiment, the first region of linked nucleosides and/or at least one flanking region may comprise at least on RBD. As a non-limiting example, the first region of linked nucleosides may comprise a RBD related to splicing factors and at least one flanking region may comprise a RBD for stability and/or translation factors.

[00198] In one embodiment, the nucleic acids and/or mRNA of the present invention may comprise at least one RBD located in a coding and/or non-coding region of the nucleic acids and/or mRNA.

[00199] In one embodiment, at least one RBD may be incorporated into at least one flanking region to increase the stability of the nucleic acid and/or mRNA of the present invention.

[00200] In one embodiment, a microRNA sequence in a RNA binding protein motif may be used to decrease the accessibility of the site of translation initiation such as, but not limited to a start codon. The signal-sensor polynucleotdies of the present invention may comprise a microRNA sequence, instead of the LNA or EJC sequence described by Matsuda et al, near the site of translation initiation in order to decrease the accessibility to the site of translation initiation. The site of translation initiation may be prior to, after or within the microRNA sequence. As a non-limiting example, the site of translation initiation may be located within a microRNA sequence such as a seed sequence or binding site. As another non-limiting example, the site of translation initiation may be located within a miR-122 sequence such as the seed sequence or the mir-122 binding site.

[00201] In another embodiment, an antisense locked nucleic acid (LNA) oligonucleotides and exon-junctino complexes (EJCs) may be used in the RNA binding protein motif. The LNA and EJCs may be used around a start codon (-4 to +37 where the A of the AUG codons is +1) in order to decrease the accessibility to the first start codon (AUG).

3' UTR and the AU Rich Elements

[00202] 3'UTRs are known to have stretches of Adenosines and Uridines embedded in them. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. C-Myc and MyoD contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Molecules containing this type of AREs include GM-CSF and TNF-a. Class III ARES are less well defined. These U rich regions do not contain an AUUUA motif. c-Jun and Myogenin are two well-studied examples of this class. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3' UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message *in vivo.*

[00203] Introduction, removal or modification of 3' UTR AU rich elements (AREs) can be used to modulate the stability of signal-sensor polynucleotides, primary constructs or mmRNA of the invention. When engineering specific polynucleotides, primary constructs or mmRNA, one or more copies of an ARE can be introduced to make polynucleotides, primary constructs or mmRNA of the invention less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein. Transfection experiments can be conducted in relevant cell lines, using signal-sensor polynucleotides, primary constructs or mmRNA of the invention and protein production can be assayed at various time points post-transfection. For example, cells can be transfected with different ARE-engineering molecules and by using an ELISA kit to the relevant protein and assaying protein produced at 6 hr, 12 hr, 24 hr, 48 hr, and 7 days post-transfection.

3 ' UTR and Triple Helices

[00204] In one embodiment, signal-sequence polynucleotides of the present invention may include a triple helix on the 3' end of the signal-sequence polynucleotides. The 3'

end of the nucleic acids of the present invention may include a triple helix alone or in combination with a Poly-A tail.

[00205] In one embodiment, the signal-sequence polynucleotides of the present invention may comprise at least a first and a second U-rich region, a conserved stem loop region between the first and second region and an A-rich region. The first and second U-rich region and the A-rich region may associate to form a triple helix on the 3' end of the nucleic acid. This triple helix may stabilize the nucleic acid, enhance the translational efficiency of the nucleic acid and/or protect the 3' end from degradation. Exemplary triple helices include, but are not limited to, the triple helix sequence of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), MEN -β and polyadenylated nuclear (PAN) RNA (See Wilusz et al, Genes & Development 2012 26:2392-2407; herein incorporated by reference in its entirety). In one embodiment, the 3' end of the modified nucleic acids, enhanced modified RNA or ribonucleic acids of the present invention comprises a first U-rich region comprising TTTTTCTTTT (SEQ ID NO: 1), a second U-rich region comprising TTTTGCTTTTT (SEQ ID NO: 2) or TTTTGCTTTT (SEQ ID NO: 3), an A-rich region comprising AAAAAGCAAAA (SEQ ID NO: 4). In another embodiment, the 3' end of the nucleic acids of the present invention comprises a triple helix formation structure comprising a first U-rich region, a conserved region, a second U-rich region and an A-rich region.

[00206] In one embodiment, the triple helix may be formed from the cleavage of a MALAT1 sequence prior to the cloverleaf structure. While not meaning to be bound by theory, MALAT1 is a long non-coding RNA which, when cleaved, forms a triple helix and a tRNA-like cloverleaf structure. The MALAT1 transcript then localizes to nuclear speckles and the tRNA-like cloverleaf localizes to the cytoplasm (Wilusz et al. Cell 2008 135(5): 919-932; herein incorporated by reference in its entirety).

[00207] As a non-limiting example, the terminal end of the nucleic acid of the present invention comprising the MALAT1 sequence can then form a triple helix structure, after RNaseP cleavage from the cloverleaf structure, which stabilizes the nucleic acid (Peart et al. *Non-mRNA 3' endformation: how the other half lives;* WIREs RNA 2013; herein incorporated by reference in its entirety).

[00208] In one embodiment, the signal-sequence polynucleotides described herein comprise a MALAT1 sequence. In another embodiment, the signal-sequence polynucleotides may be polyadenylated. In yet another embodiment, the signal-sequence polynucleotides is not polyadenylated but has an increased resistance to degradation compared to unmodified nucleic acids or mRNA.

[00209] In one embodiment, the signal-sequence polynucleotides of the present invention may comprise a MALAT1 sequence in the second flanking region (e.g., the 3'UTR). As a non-limiting example, the MALAT1 sequence may be human or mouse.

[00210] In another embodiment, the cloverleaf structure of the MALAT1 sequence may also undergo processing by RNaseZ and CCA adding enzyme to form a tRNA-like structure called mascRNA (MALAT1-associated small cytoplasmic RNA). As a non-limiting example, the mascRNA may encode a protein or a fragment thereof and/or may comprise a microRNA sequence. The mascRNA may comprise at least one chemical modification described herein.

Stem Loop

[00211] In one embodiment, the nucleic acids of the present invention may include a stem loop such as, but not limited to, a histone stem loop. The stem loop may be a nucleotide sequence that is about 25 or about 26 nucleotides in length such as, but not limited to, SEQ ID NOs: 7-17 as described in International Patent Publication No. WO2013 103659, herein incorporated by reference in its entirety. The histone stem loop may be located 3' relative to the coding region (e.g., at the 3' terminus of the coding region). As a non-limiting example, the stem loop may be located at the 3' end of a nucleic acid described herein.

[00212] In one embodiment, the stem loop may be located in the second terminal region. As a non-limiting example, the stem loop may be located within an untranslated region (e.g., 3'UTR) in the second terminal region.

[00213] In one embodiment, the nucleic acid such as, but not limited to mRNA, which comprises the histone stem loop may be stabilized by the addition of at least one chain terminating nucleoside. Not wishing to be bound by theory, the addition of at least one chain terminating nucleoside may slow the degradation of a nucleic acid and thus can increase the half-life of the nucleic acid.

[00214] In one embodiment, the chain terminating nucleoside may be, but is not limited to, those described in International Patent Publication No. WO2013 103659, herein incorporated by reference in its entirety. In another embodiment, the chain terminating nucleosides which may be used with the present invention includes, but is not limited to, 3'-deoxyadenosine (cordycepin), 3'-deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, 2',3'-dideoxynucleosides, such as 2',3'-dideoxyadenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'- dideoxyguanosine, 2',3'-dideoxythymine, a 2'-deoxynucleoside, or a -O- methylnucleoside.

[00215] In another embodiment, the nucleic acid such as, but not limited to mRNA, which comprises the histone stem loop may be stabilized by a modification to the 3'region of the nucleic acid that can prevent and/or inhibit the addition of oligio(U) (see e.g., International Patent Publication No. WO2013 103659, herein incorporated by reference in its entirety).

[00216] In yet another embodiment, the nucleic acid such as, but not limited to mRNA, which comprises the histone stem loop may be stabilized by the addition of an oligonucleotide that terminates in a 3'-deoxynucleoside, 2',3'-dideoxynucleoside 3'-0~ methy!nucieosides, 3'-0-ethylnucleosides, 3'-arabmosides, and other modified nucleosides known in the art and/or described herein.

[00217] In one embodiment, the nucleic acids of the present invention may include a histone stem loop, a polyA tail sequence and/or a 5'cap structure. The histone stem loop may be before and/or after the polyA tail sequence. The nucleic acids comprising the histone stem loop and a polyA tail sequence may include a chain terminating nucleoside described herein.

[00218] In another embodiment, the nucleic acids of the present invention may include a histone stem loop and a 5'cap structure. The 5'cap structure may include, but is not limited to, those described herein and/or known in the art.

[00219] In one embodiment, the conserved stem loop region may comprise a miR sequence described herein. As a non-limiting example, the stem loop region may comprise the seed sequence of a miR sequence described herein. In another non-limiting example, the stem loop region may comprise a miR- 122 seed sequence.

[00220] In another embodiment, the conserved stem loop region may comprise a miR sequence described herein and may also include a TEE sequence.

[00221] In one embodiment, the incorporation of a miR sequence and/or a TEE sequence changes the shape of the stem loop region which may increase and/or descrease translation. (see e.g, Kedde et al. A Pumilio-induced RNA structure switch in p27-3'UTR controls miR-221 and miR-22 accessibility. Nature Cell Biology. 2010, herein incorporated by reference in its entirety).

5' Capping

[00222] The 5' cap structure of an mRNA is involved in nuclear export, increasing mRNA stability and binds the mRNA Cap Binding Protein (CBP), which is responsibile for mRNA stability in the cell and translation competency through the association of CBP with poly(A) binding protein to form the mature cyclic mRNA species. The cap further assists the removal of 5' proximal introns removal during mRNA splicing.

[00223] Endogenous mRNA molecules may be 5'-end capped generating a 5'-ppp-5'-triphosphate linkage between a terminal guanosine cap residue and the 5'-terminal transcribed sense nucleotide of the mRNA molecule. This 5'-guanylate cap may then be methylated to generate an N7-methyl-guanylate residue. The ribose sugars of the terminal and/or anteterminal transcribed nucleotides of the 5' end of the mRNA may optionally also be 2'-0-methylated. 5'-decapping through hydrolysis and cleavage of the guanylate cap structure may target a nucleic acid molecule, such as an mRNA molecule, for degradation.

[00224] Modifications to the signal-sensor polynucleotides, primary constructs, and mmRNA of the present invention may generate a non-hydrolyzable cap structure preventing decapping and thus increasing mRNA half-life. Because cap structure hydrolysis requires cleavage of 5'-ppp-5' phosphorodiester linkages, modified nucleotides may be used during the capping reaction. For example, a Vaccinia Capping Enzyme from New England Biolabs (Ipswich, MA) may be used with a-thio-guanosine nucleotides according to the manufacturer's instructions to create a phosphorothioate linkage in the 5'-ppp-5' cap. Additional modified guanosine nucleotides may be used such as a-methyl-phosphonate and seleno-phosphate nucleotides.

[00225] Additional modifications include, but are not limited to, 2'-0-methylation of the ribose sugars of 5'-terminal and/or 5'-anteterminal nucleotides of the mRNA (as mentioned above) on the 2'-hydroxyl group of the sugar ring. Multiple distinct 5'-cap structures can be used to generate the 5'-cap of a nucleic acid molecule, such as an mRNA molecule.

[00226] Cap analogs, which herein are also referred to as synthetic cap analogs, chemical caps, chemical cap analogs, or structural or functional cap analogs, differ from natural (i.e. endogenous, wild-type or physiological) 5'-caps in their chemical structure, while retaining cap function. Cap analogs may be chemically (i.e. non-enzymatically) or enzymatically synthesized and/linked to a nucleic acid molecule.

[00227] For example, the Anti-Reverse Cap Analog (ARCA) cap contains two guanines linked by a 5'-5'-triphosphate group, wherein one guanine contains an N7 methyl group as well as a 3'-0-methyl group (i.e., N7,3'-0-dimethyl-guanosine-5'-triphosphate-5'-guanosine (m^7G-3'mppp-G; which may equivaliently be designated 3' O-Me-m7G(5')ppp(5')G). The 3'-0 atom of the other, unmodified, guanine becomes linked to the 5'-terminal nucleotide of the capped nucleic acid molecule (e.g. an mRNA or mmRNA). The N7- and 3'-0-methlyated guanine provides the terminal moiety of the capped nucleic acid molecule (e.g. mRNA or mmRNA).

[00228] Another exemplary cap is mCAP, which is similar to ARCA but has a 2'-0-methyl group on guanosine (i.e., N7,2'-0-dimethyl-guanosine-5'-triphosphate-5'-guanosine, m^7Gm-ppp-G).

[00229] While cap analogs allow for the concomitant capping of a nucleic acid molecule in an in vitro transcription reaction, up to 20% of transcripts remain uncapped. This, as well as the structural differences of a cap analog from an endogenous 5'-cap structures of nucleic acids produced by the endogenous, cellular transcription machinery, may lead to reduced translational competency and reduced cellular stability.

[00230] Signal-sensor polynucleotides, primary constructs and mmRNA of the invention may also be capped post-transcriptionally, using enzymes, in order to generate more authentic 5'-cap structures. As used herein, the phrase "more authentic" refers to a feature that closely mirrors or mimics, either structurally or functionally, an endogenous or wild type feature. That is, a "more authentic" feature is better representative of an

endogenous, wild-type, natural or physiological cellular function and/or structure as compared to synthetic features or analogs, etc., of the prior art, or which outperforms the corresponding endogenous, wild-type, natural or physiological feature in one or more respects. Non-limiting examples of more authentic 5'cap structures of the present invention are those which, among other things, have enhanced binding of cap binding proteins, increased half life, reduced susceptibility to 5' endonucleases and/or reduced 5'decapping, as compared to synthetic 5'cap structures known in the art (or to a wild-type, natural or physiological 5'cap structure). For example, recombinant Vaccinia Virus Capping Enzyme and recombinant 2'-0-methyltransferase enzyme can create a canonical 5'-5'-triphosphate linkage between the 5'-terminal nucleotide of an mRNA and a guanine cap nucleotide wherein the cap guanine contains an N7 methylation and the 5'-terminal nucleotide of the mRNA contains a 2'-0-methyl. Such a structure is termed the Capl structure. This cap results in a higher translational-competency and cellular stability and a reduced activation of cellular pro-inflammatory cytokines, as compared, e.g., to other 5'cap analog structures known in the art. Cap structures include 7mG(5')ppp(5')N,pN2p (cap 0), 7mG(5')ppp(5')NlmpNp (cap 1), and 7mG(5')-ppp(5')NlmpN2mp (cap 2).

[00231] Because the signal-sensor polynucleotides, primary constructs or mmRNA may be capped post-transcriptionally, and because this process is more efficient, nearly 100% of the signal-sensor polynucleotides, primary constructs or mmRNA may be capped. This is in contrast to -80% when a cap analog is linked to an mRNA in the course of an *in vitro* transcription reaction.

[00232] According to the present invention, 5' terminal caps may include endogenous caps or cap analogs. According to the present invention, a 5' terminal cap may comprise a guanine analog. Useful guanine analogs include inosine, Nl-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

Viral Sequences

[00233] Additional viral sequences such as, but not limited to, the translation enhancer sequence of the barley yellow dwarf virus (BYDV-PAV) can be engineered and inserted in the 3' UTR of the signal-sensor polynucleotides, primary constructs or mmRNA of the invention and can stimulate the translation of the construct *in vitro* and *in vivo.*

Transfection experiments can be conducted in relevant cell lines at and protein production can be assayed by ELISA at 12hr, 24hr, 48hr, 72 hr and day 7 post-transfection.

IRES Sequences

[00234] Further, provided are signal-sensor polynucleotides, primary constructs or mmRNA which may contain an internal ribosome entry site (IRES). First identified as a feature Picorna virus RNA, IRES plays an important role in initiating protein synthesis in absence of the 5' cap structure. An IRES may act as the sole ribosome binding site, or may serve as one of multiple ribosome binding sites of an mRNA. signal-sensor polynucleotides, primary constructs or mmRNA containing more than one functional ribosome binding site may encode several oncology-related peptides or oncology-related polypeptides that are translated independently by the ribosomes ("multicistronic nucleic acid molecules"). When signal-sensor polynucleotides, primary constructs or mmRNA are provided with an IRES, further optionally provided is a second translatable region. Examples of IRES sequences that can be used according to the invention include without limitation, those from picornaviruses (e.g. FMDV), pest viruses (CFFV), polio viruses (PV), encephalomyocarditis viruses (ECMV), foot-and-mouth disease viruses (FMDV), hepatitis C viruses (HCV), classical swine fever viruses (CSFV), murine leukemia virus (MLV), simian immune deficiency viruses (SIV) or cricket paralysis viruses (CrPV).

Poly-A tails

[00235] During RNA processing, a long chain of adenine nucleotides (poly-A tail) may be added to a polynucleotide such as an mRNA molecule in order to increase stability. Immediately after transcription, the 3' end of the transcript may be cleaved to free a 3' hydroxyl. Then poly-A polymerase adds a chain of adenine nucleotides to the RNA. The process, called polyadenylation, adds a poly-A tail that can be between 100 and 250 residues long.

[00236] It has been discovered that unique poly-A tail lengths provide certain advantages to the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention.

[00237] Generally, the length of a poly-A tail of the present invention is greater than 30 nucleotides in length. In another embodiment, the poly-A tail is greater than 35

nucleotides in length (e.g., at least or greater than about 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,500, and 3,000 nucleotides). In some embodiments, the signal-sensor polynucleotides, primary construct, or mmRNA includes from about 30 to about 3,000 nucleotides (e.g., from 30 to 50, from 30 to 100, from 30 to 250, from 30 to 500, from 30 to 750, from 30 to 1,000, from 30 to 1,500, from 30 to 2,000, from 30 to 2,500, from 50 to 100, from 50 to 250, from 50 to 500, from 50 to 750, from 50 to 1,000, from 50 to 1,500, from 50 to 2,000, from 50 to 2,500, from 50 to 3,000, from 100 to 500, from 100 to 750, from 100 to 1,000, from 100 to 1,500, from 100 to 2,000, from 100 to 2,500, from 100 to 3,000, from 500 to 750, from 500 to 1,000, from 500 to 1,500, from 500 to 2,000, from 500 to 2,500, from 500 to 3,000, from 1,000 to 1,500, from 1,000 to 2,000, from 1,000 to 2,500, from 1,000 to 3,000, from 1,500 to 2,000, from 1,500 to 2,500, from 1,500 to 3,000, from 2,000 to 3,000, from 2,000 to 2,500, and from 2,500 to 3,000).

[00238] In one embodiment, the poly-A tail is designed relative to the length of the overall signal-sensor polynucleotides, primary constructs or mmRNA. This design may be based on the length of the coding region, the length of a particular feature or region (such as the first or flanking regions), or based on the length of the ultimate product expressed from the polynucleotides, primary constructs or mmRNA.

[00239] In this context the poly-A tail may be 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% greater in length than the signal-sensor polynucleotides, primary constructs or mmRNA or feature thereof. The poly-A tail may also be designed as a fraction of polynucleotides, primary constructs or mmRNA to which it belongs. In this context, the poly-A tail may be 10, 20, 30, 40, 50, 60, 70, 80, or 90% or more of the total length of the construct or the total length of the construct minus the poly-A tail.

[00240] In one embodiment, engineered binding sites and/or conjugation of signal-sensor polynucleotides, primary constructs or mmRNA for Poly-A binding protein may be used to enhance expression. The engineered binding sites may be sensor sequences which can operate as binding sites for ligands of the local microenvironment of the nucleic acids and/or mRNA. As a non-limiting example, the nucleic acids and/or mRNA may comprise at least one engineered binding site to alter the binding affinity of Poly-A

binding protein (PABP) and analogs thereof. The incorporation of at least one engineered binding site may increase the binding affinity of the PABP and analogs thereof.

[00241] Additionally, multiple distinct signal-sensor polynucleotides, primary constructs or mmRNA may be linked together to the PABP (Poly-A binding protein) through the 3'-end using modified nucleotides at the 3'-terminus of the poly-A tail. Transfection experiments can be conducted in relevant cell lines and protein production can be assayed by ELISA at 12hr, 24hr, 48hr, 72 hr and day 7 post-transfection. As a non-limiting example, the transfection experiments may be used to evaluate the effect on PABP or analogs thereof binding affinity as a result of the addition of at least one engineered binding site.

[00242] In one embodiment, the signal-sensor polynucleotides and primary constructs of the present invention are designed to include a polyA-G Quartet. The G-quartet is a cyclic hydrogen bonded array of four guanine nucleotides that can be formed by G-rich sequences in both DNA and RNA. In this embodiment, the G-quartet is incorporated at the end of the poly-A tail. The resultant mmRNA construct is assayed for stability, protein production and other parameters including half-life at various time points. It has been discovered that the polyA-G quartet results in protein production equivalent to at least 75% of that seen using a poly-A tail of 120 nucleotides alone.

[00243] In one embodiment, the nucleic acids or mRNA of the present invention may comprise a polyA tail and may be stabilized by the addition of a chain terminating nucleoside. The nucleic acids and/or mRNA with a polyA tail may further comprise a 5'cap structure.

[00244] In another embodiment, the nucleic acids or mRNA of the present invention may comprise a polyA-G Quartet. The nucleic acids and/or mRNA with a polyA-G Quartet may further comprise a 5'cap structure.

[00245] In one embodiment, the chain terminating nucleoside which may be used to stabilize the nucleic acid or mRNA comprising a polyA tail or polyA-G Quartet may be, but is not limited to, those described in International Patent Publication No. WO2013103659, herein incorporated by reference in its entirety. In another embodiment, the chain terminating nucleosides which may be used with the present

invention includes, but is not limited to, 3'-deoxyadenosine (cordycepin), 3'-deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, 2',3'-dideoxynucleosides, such as 2',3'- dideoxyadenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'- dideoxyguanosine, 2',3'-dideoxythymine, a 2'-deoxynucleoside, or a -O- methylnucleoside.

[00246] In another embodiment, the nucleic acid such as, but not limited to mRNA, which comprise a polyA tail or a polyA-G Quartet may be stabilized by a modification to the 3'region of the nucleic acid that can prevent and/or inhibit the addition of oligio(U) (see e.g., International Patent Publication No. WO2013 103659, herein incorporated by reference in its entirety).

[00247] In yet another embodiment, the nucleic acid such as, but not limited to mRNA, which comprise a polyA tail or a polyA-G Quartet may be stabilized by the addition of an oligonucleotide that terminates in a 3'-deoxynucleoside, 2', 3'-dideoxynucleoside 3'-0- methylnucSeosides, 3'~Q~eihyimic]eosides, 3'-arabinosides, and other modified nucleosides known in the art and/or described herein.

Quantification

[00248] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be quantified in exosomes derived from one or more bodily fluid. As used herein "bodily fluids" include peripheral blood, serum, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyl cavity fluid, and umbilical cord blood. Alternatively, exosomes may be retrieved from an organ selected from the group consisting of lung, heart, pancreas, stomach, intestine, bladder, kidney, ovary, testis, skin, colon, breast, prostate, brain, esophagus, liver, and placenta.

[00249] In the quantification method, a sample of not more than 2mL is obtained from the subject and the exosomes isolated by size exclusion chromatography, density gradient

centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfiuidic separation, or combinations thereof. In the analysis, the level or concentration of signal-sensor polynucleotides, primary construct or mmRNA may be an expression level, presence, absence, truncation or alteration of the administered construct. It is advantageous to correlate the level with one or more clinical phenotypes or with an assay for a human disease biomarker. The assay may be performed using construct specific probes, cytometry, qRT-PCR, real-time PCR, PCR, flow cytometry, electrophoresis, mass spectrometry, or combinations thereof while the exosomes may be isolated using immunohistochemical methods such as enzyme linked immunosorbent assay (ELISA) methods. Exosomes may also be isolated by size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfiuidic separation, or combinations thereof.

[00250] These methods afford the investigator the ability to monitor, in real time, the level of signal-sensor polynucleotides, primary constructs or mmRNA remaining or delivered. This is possible because the polynucleotides, primary constructs or mmRNA of the present invention differ from the endogenous forms due to the structural and/or chemical modifications.

II. Design and synthesis of signal-sensor polynucleotides

[00251] Signal-sensor polynucleotides, primary constructs or mmRNA for use in accordance with the invention may be prepared according to any available technique including, but not limited to chemical synthesis, enzymatic synthesis, which is generally termed *in vitro* transcription (IVT) or enzymatic or chemical cleavage of a longer precursor, etc. Methods of synthesizing RNAs are known in the art (see, *e.g.*, Gait, M.J. (ed.) *Oligonucleotide synthesis: a practical approach,* Oxford [Oxfordshire], Washington, DC: IRL Press, 1984; and Herdewijn, P. (ed.) *Oligonucleotide synthesis: methods and applications,* Methods in Molecular Biology, v. 288 (Clifton, N.J.) Totowa, N.J.: Humana Press, 2005; both of which are incorporated herein by reference).

[00252] The process of design and synthesis of the signal-sensor primary constructs of the invention generally includes the steps of gene construction, mRNA production (either with or without modifications) and purification. In the enzymatic synthesis method, a

target signal-sensor polynucleotide sequence encoding the oncology-related polypeptide of interest is first selected for incorporation into a vector which will be amplified to produce a cDNA template. Optionally, the target signal-sensor polynucleotide sequence and/or any flanking sequences may be codon optimized. The cDNA template is then used to produce mRNA through *in vitro* transcription (IVT). After production, the mRNA may undergo purification and clean-up processes. The steps of which are provided in more detail below.

Gene Construction

[00253] The step of gene construction may include, but is not limited to gene synthesis, vector amplification, plasmid purification, plasmid linearization and clean-up, and cDNA template synthesis and clean-up.

Gene Synthesis

[00254] Once an oncology-related polypeptide of interest, or target, is selected for production, a signal-sensor primary construct is designed. Within the primary construct, a first region of linked nucleosides encoding the polypeptide of interest may be constructed using an open reading frame (ORF) of a selected nucleic acid (DNA or RNA) transcript. The ORF may comprise the wild type ORF, an isoform, variant or a fragment thereof. As used herein, an "open reading frame" or "ORF" is meant to refer to a nucleic acid sequence (DNA or RNA) which is capable of encoding an oncology-related polypeptide of interest. ORFs often begin with the start codon, ATG and end with a nonsense or termination codon or signal.

[00255] Further, the nucleotide sequence of the first region may be codon optimized. Codon optimization methods are known in the art and may be useful in efforts to achieve one or more of several goals. These goals include to match codon frequencies in target and host organisms to ensure proper folding, bias GC content to increase mRNA stability or reduce secondary structures, minimize tandem repeat codons or base runs that may impair gene construction or expression, customize transcriptional and translational control regions, insert or remove protein trafficking sequences, remove/add post translation modification sites in encoded protein (e.g. glycosylation sites), add, remove or shuffle protein domains, insert or delete restriction sites, modify ribosome binding sites and mRNA degradation sites, to adjust translational rates to allow the various domains of

the protein to fold properly, or to reduce or eliminate problem secondary structures within the **mPvNA.** Codon optimization tools, algorithms and services are known in the art, non-limiting examples include services from GeneArt (Life Technologies) and/or DNA2**.0** (Menlo Park CA). In one embodiment, the ORF sequence is optimized using optimization algorithms. Codon options for each amino acid are given in Table 1.

Table 1. Codon Options

Amino Acid	Single Letter Code	Codon Options
Isoleucine	I	ATT, ATC, ATA
Leucine	L	CTT, CTC, CTA, CTG, TTA, TTG
Valine	V	GTT, GTC, GTA, GTG
Phenylalanine	F	TTT, TTC
Methionine	M	ATG
Cysteine	C	TGT, TGC
Alanine	A	GCT, GCC, GCA, GCG
Glycine	G	GGT, GGC, GGA, GGG
Proline	P	CCT, CCC, CCA, CCG
Threonine	T	ACT, ACC, ACA, ACG
Serine	S	TCT, TCC, TCA, TCG, AGT, AGC
Tyrosine	Y	TAT, TAC
Tryptophan	W	TGG
Glutamine	Q	CAA, CAG
Asparagine	N	AAT, AAC
Histidine	H	CAT, CAC
Glutamic acid	E	GAA, GAG
Aspartic acid	D	GAT, GAC
Lysine	K	AAA, AAG
Arginine	R	CGT, CGC, CGA, CGG, AGA, AGG
Selenocysteine	Sec	UGA in mRNA in presence of Selenocystein insertion element (SECIS)
Stop codons	Stop	TAA, TAG, TGA

[00256] In one embodiment, after a nucleotide sequence has been codon optimized it may be further evaluated for regions containing restriction sites. At least one nucleotide within the restriction site regions may be replaced with another nucleotide in order to remove the restriction site from the sequence but the replacement of nucleotides does alter the amino acid sequence which is encoded by the codon optimized nucleotide sequence.

[00257] Features, which may be considered beneficial in some embodiments of the present invention, may be encoded by the signal-sensor primary construct and may flank the ORF as a first or second flanking region. The flanking regions may be incorporated

into the signal-sensor primary construct before and/or after optimization of the ORF. It is not required that a signal-sensor primary construct contain both a 5' and 3' flanking region. Examples of such features include, but are not limited to, untranslated regions (UTRs), Kozak sequences, an oligo(dT) sequence, and detectable tags and may include multiple cloning sites which may have Xbal recognition.

[00258] In some embodiments, a 5' UTR and/or a 3' UTR may be provided as flanking regions. Multiple 5' or 3' UTRs may be included in the flanking regions and may be the same or of different sequences. Any portion of the flanking regions, including none, may be codon optimized and any may independently contain one or more different structural or chemical modifications, before and/or after codon optimization. Combinations of features may be included in the first and second flanking regions and may be contained within other features. For example, the ORF may be flanked by a 5' UTR which may contain a strong Kozak translational initiation signal and/or a 3' UTR which may include an oligo(dT) sequence for templated addition of a poly-A tail.

[00259] Tables 2 and 3 provide a listing of exemplary UTRs which may be utilized in the signal-sensor primary construct of the present invention as flanking regions. Shown in Table 2 is a representative listing of a 5'-untranslated region of the invention. Variants of 5' UTRs may be utilized wherein one or more nucleotides are added or removed to the termini, including A, T, C or G.

Table 2. 5'-Untranslated Regions

5′ UTR Identifier	Name/ Description	Sequence	SEQ ID NO.
Native	Wild type UTR	See wild type sequence	-
5UTR-001	Synthetic UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGA AGAAATATAAGAGCCACC	1
5UTR-002	Upstream UTR	GGGAGATCAGAGAGAAAAGAAGAGTAAGA AGAAATATAAGAGCCACC	2
5UTR-003	Upstream UTR	GGAATAAAAGTCTCAACACAACATATACAA AACAAACGAATCTCAAGCAATCAAGCATTC TACTTCTATTGCAGCAATTTAAATCATTTCT TTTAAAGCAAAAGCAATTTTCTGAAAATTT TCACCATTTACGAACGATAGCAAC	3
5UTR-004	Upstream UTR	GGGAGACAAGCUUGGCAUUCCGGUACUGU UGGUAAAGCCACC	4

[00260] Shown in Table 3 is a representative listing of 3'-untranslated regions of the invention. Variants of 3' UTRs may be utilized wherein one or more nucleotides are added or removed to the termini, including A, T, C or G.

Table 3. 3'-Untranslated Regions

3′ UTR Identifier	Name/ Description	Sequence	SEQ ID NO.
3UTR-001	Creatine Kinase	GCGCCTGCCCACCTGCCACCGACTGCTGGAACC CAGCCAGTGGGAGGGCCTGGCCCACCAGAGTCC TGCTCCCTCACTCCTCGCCCCGCCCCCTGTCCCA GAGTCCCACCTGGGGGCTCTCTCCACCCTTCTCA GAGTTCCAGTTTCAACCAGAGTTCCAACCAATG GGCTCCATCCTCTGGATTCTGGCCAATGAAATAT CTCCCTGGCAGGGTCCTCTTCTTTTCCCAGAGCT CCACCCCAACCAGGAGCTCTAGTTAATGGAGAG CTCCCAGCACACTCGGAGCTTGTGCTTTGTCTCC ACGCAAAGCGATAAATAAAAGCATTGGTGGCCT TTGGTCTTTGAATAAAGCCTGAGTAGGAAGTCTA GA	5
3UTR-002	Myoglobin	GCCCCTGCCGCTCCCACCCCCACCCATCTGGGCC CCGGGTTCAAGAGAGAGCGGGGTCTGATCTCGT GTAGCCATATAGAGTTTGCTTCTGAGTGTCTGCT TTGTTTAGTAGAGGTGGGCAGGAGGAGCTGAGG GGCTGGGGCTGGGGTGTTGAAGTTGGCTTTGCAT GCCCAGCGATGCGCCTCCCTGTGGGATGTCATCA CCCTGGGAACCGGGAGTGGCCCTTGGCTCACTG TGTTCTGCATGGTTTGGATCTGAATTAATTGTCC TTTCTTCTAAATCCCAACCGAACTTCTTCCAACC TCCAAACTGGCTGTAACCCCAAATCCAAGCCATT AACTACACCTGACAGTAGCAATTGTCTGATTAAT CACTGGCCCCTTGAAGACAGCAGAATGTCCCTTT GCAATGAGGAGGAGATCTGGGCTGGGCGGGCCA GCTGGGGAAGCATTTGACTATCTGGAACTTGTGT GTGCCTCCTCAGGTATGGCAGTGACTCACCTGGT TTTAATAAAACAACCTGCAACATCTCATGGTCTT TGAATAAAGCCTGAGTAGGAAGTCTAGA	6
3UTR-003	α-actin	ACACACTCCACCTCCAGCACGCGACTTCTCAGG ACGACGAATCTTCTCAATGGGGGGGCGGCTGAG CTCCAGCCACCCCGCAGTCACTTTCTTTGTAACA ACTTCCGTTGCTGCCATCGTAAACTGACACAGTG TTTATAACGTGTACATACATTAACTTATTACCTC ATTTTGTTATTTTTCGAAACAAAGCCCTGTGGAA GAAAATGGAAAACTTGAAGAAGCATTAAAGTCA TTCTGTTAAGCTGCGTAAATGGTCTTTGAATAAA GCCTGAGTAGGAAGTCTAGA	7
3UTR-004	Albumin	CATCACATTTAAAAGCATCTCAGCCTACCATGAG AATAAGAGAAAGAAAATGAAGATCAAAAGCTT ATTCATCTGTTTTTCTTTTTCGTTGGTGTAAAGCC	8

		AACACCCTGTCTAAAAAACATAAATTTCTTTAAT CATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAA AAAATGGAAAGAATCTAATAGAGTGGTACAGCA CTGTTATTTTTCAAAGATGTGTTGCTATCCTGAA AATTCTGTAGGTTCTGTGGAAGTTCCAGTGTTCT CTCTTATTCCACTTCGGTAGAGGATTTCTAGTTT CTTGTGGGCTAATTAAATAAATCATTAATACTCT TCTAATGGTCTTTGAATAAAGCCTGAGTAGGAA GTCTAGA	
3UTR-005	α-globin	GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGC CCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTC TTTGAATAAAGCCTGAGTAGGAAGGCGGCCGCT CGAGCATGCATCTAGA	9
3UTR-006	G-CSF	GCCAAGCCCTCCCCATCCCATGTATTTATCTCTA TTTAATATTTATGTCTATTTAAGCCTCATATTTAA AGACAGGGAAGAGCAGAACGGAGCCCCAGGCC TCTGTGTCCTTCCCTGCATTTCTGAGTTTCATTCT CCTGCCTGTAGCAGTGAGAAAAAGCTCCTGTCCT CCCATCCCCTGGACTGGGAGGTAGATAGGTAAA TACCAAGTATTTATTACTATGACTGCTCCCCAGC CCTGGCTCTGCAATGGGCACTGGGATGAGCCGC TGTGAGCCCCTGGTCCTGAGGGTCCCCACCTGGG ACCCTTGAGAGTATCAGGTCTCCCACGTGGGAG ACAAGAAATCCCTGTTTAATATTTAAACAGCAGT GTTCCCCATCTGGGTCCTTGCACCCCTCACTCTG GCCTCAGCCGACTGCACAGCGGCCCCTGCATCC CCTTGGCTGTGAGGCCCCTGGACAAGCAGAGGT GGCCAGAGCTGGGAGGCATGGCCCTGGGGTCCC ACGAATTTGCTGGGGAATCTCGTTTTTCTTCTTA AGACTTTTGGGACATGGTTTGACTCCCGAACATC ACCGACGCGTCTCCTGTTTTTCTGGGTGGCCTCG GGACACCTGCCCTGCCCCCACGAGGGTCAGGAC TGTGACTCTTTTTAGGGCCAGGCAGGTGCCTGGA CATTTGCCTTGCTGGACGGGGACTGGGGATGTG GGAGGGAGCAGACAGGAGGAATCATGTCAGGC CTGTGTGTGAAAGGAAGCTCCACTGTCACCCTCC ACCTCTTCACCCCCCACTCACCAGTGTCCCCTCC ACTGTCACATTGTAACTGAACTTCAGGATAATAA AGTGTTTGCCTCCATGGTCTTTGAATAAAGCCTG AGTAGGAAGGCGGCCGCTCGAGCATGCATCTAG A	10
3UTR-007	Colla2; collagen, type I, alpha 2	ACTCAATCTAAATTAAAAAAGAAAGAAATTTGA AAAAACTTTCTCTTTGCCATTTCTTCTTCTTCTTT TTTAACTGAAAGCTGAATCCTTCCATTTCTTCTG CACATCTACTTGCTTAAATTGTGGGCAAAAGAG AAAAAGAAGGATTGATCAGAGCATTGTGCAATA CAGTTTCATTAACTCCTTCCCCCGCTCCCCCAAA AATTTGAATTTTTTTTTCAACACTCTTACACCTGT TATGGAAAATGTCAACCTTTGTAAGAAAACCAA AATAAAAATTGAAAAATAAAAACCATAAACATT TGCACCACTTGTGGCTTTTGAATATCTTCCACAG	11

		AGGGAAGTTTAAAACCCAAACTTCCAAAGGTTT AAACTACCTCAAAACACTTTCCCATGAGTGTGAT CCACATTGTTAGGTGCTGACCTAGACAGAGATG AACTGAGGTCCTTGTTTTGTTTTGTTCATAATAC AAAGGTGCTAATTAATAGTATTTCAGATACTTGA AGAATGTTGATGGTGCTAGAAGAATTTGAGAAG AAATACTCCTGTATTGAGTTGTATCGTGTGGTGT ATTTTTTAAAAAATTTGATTTAGCATTCATATTTT CCATCTTATTCCCAATTAAAAGTATGCAGATTAT TTGCCCAAATCTTCTTCAGATTCAGCATTTGTTCT TTGCCAGTCTCATTTTCATCTTCTTCCATGGTTCC ACAGAAGCTTTGTTTCTTGGGCAAGCAGAAAAA TTAAATTGTACCTATTTTGTATATGTGAGATGTT TAAATAAATTGTGAAAAAAATGAAATAAAGCAT GTTTGGTTTTCCAAAAGAACATAT	
3UTR-008	Col6a2; collagen, type VI, alpha 2	CGCCGCCGCCCGGGCCCCGCAGTCGAGGGTCGT GAGCCCACCCCGTCCATGGTGCTAAGCGGGCCC GGGTCCCACACGGCCAGCACCGCTGCTCACTCG GACGACGCCCTGGGCCTGCACCTCTCCAGCTCCT CCCACGGGGTCCCCGTAGCCCCGGCCCCCGCCC AGCCCCAGGTCTCCCCAGGCCCTCCGCAGGCTG CCCGGCCTCCCTCCCCCTGCAGCCATCCCAAGGC TCCTGACCTACCTGGCCCCTGAGCTCTGGAGCAA GCCCTGACCCAATAAAGGCTTTGAACCCAT	12
3UTR-009	RPN1; ribophorin I	GGGGCTAGAGCCCTCTCCGCACAGCGTGGAGAC GGGGCAAGGAGGGGGGTTATTAGGATTGGTGGT TTTGTTTTGCTTTGTTTAAAGCCGTGGGAAAATG GCACAACTTTACCTCTGTGGGAGATGCAACACT GAGAGCCAAGGGGTGGGAGTTGGGATAATTTTT ATATAAAAGAAGTTTTTCCACTTTGAATTGCTAA AAGTGGCATTTTTCCTATGTGCAGTCACTCCTCT CATTTCTAAAATAGGGACGTGGCCAGGCACGGT GGCTCATGCCTGTAATCCCAGCACTTTGGGAGGC CGAGGCAGGCGGCTCACGAGGTCAGGAGATCGA GACTATCCTGGCTAACACGGTAAAACCCTGTCTC TACTAAAAGTACAAAAAATTAGCTGGGCGTGGT GGTGGGCACCTGTAGTCCCAGCTACTCGGGAGG CTGAGGCAGGAGAAAGGCATGAATCCAAGAGG CAGAGCTTGCAGTGAGCTGAGATCACGCCATTG CACTCCAGCCTGGGCAACAGTGTTAAGACTCTGT CTCAAATATAAATAAATAAATAAATAAATAAAT AAATAAATAAAAATAAAGCGAGATGTTGCCCTC AAA	13
3UTR-010	LRP1; low density lipoprotein receptor-related protein 1	GGCCCTGCCCCGTCGGACTGCCCCCAGAAAGCC TCCTGCCCCCTGCCAGTGAAGTCCTTCAGTGAGC CCCTCCCCAGCCAGCCCTTCCCTGGCCCCGCCGG ATGTATAAATGTAAAAATGAAGGAATTACATTT TATATGTGAGCGAGCAAGCCGGCAAGCGAGCAC AGTATTATTTCTCCATCCCCTCCCTGCCTGCTCCT TGGCACCCCCATGCTGCCTTCAGGGAGACAGGC AGGGAGGGCTTGGGGCTGCACCTCCTACCCTCC	14

		CACCAGAACGCACCCCACTGGGAGAGCTGGTGG TGCAGCCTTCCCCTCCCTGTATAAGACACTTTGC CAAGGCTCTCCCCTCTCGCCCCATCCCTGCTTGC CCGCTCCCACAGCTTCCTGAGGGCTAATTCTGGG AAGGGAGAGTTCTTTGCTGCCCCTGTCTGGAAG ACGTGGCTCTGGGTGAGGTAGGCGGGAAAGGAT GGAGTGTTTTAGTTCTTGGGGGAGGCCACCCCA AACCCCAGCCCCAACTCCAGGGGCACCTATGAG ATGGCCATGCTCAACCCCCCTCCCAGACAGGCC CTCCCTGTCTCCAGGGCCCCCACCGAGGTTCCCA GGGCTGGAGACTTCCTCTGGTAAACATTCCTCCA GCCTCCCCTCCCCTGGGGACGCCAAGGAGGTGG GCCACACCCAGGAAGGGAAAGCGGGCAGCCCC GTTTTGGGGACGTGAACGTTTTAATAATTTTTGC TGAATTCCTTTACAACTAAATAACACAGATATTG TTATAAATAAAATTGT	
3UTR-01 1	Nntl; cardiotrophin-like cytokine factor 1	ATATTAAGGATCAAGCTGTTAGCTAATAATGCC ACCTCTGCAGTTTTGGGAACAGGCAAATAAAGT ATCAGTATACATGGTGATGTACATCTGTAGCAA AGCTCTTGGAGAAAATGAAGACTGAAGAAAGCA AAGCAAAAACTGTATAGAGAGATTTTTCAAAAG CAGTAATCCCTCAATTTTAAAAAAGGATTGAAA ATTCTAAATGTCTTTCTGTGCATATTTTTTGTGTT AGGAATCAAAAGTATTTTATAAAAGGAGAAAGA ACAGCCTCATTTTAGATGTAGTCCTGTTGGATTT TTTATGCCTCCTCAGTAACCAGAAATGTTTTAAA AAACTAAGTGTTTAGGATTTCAAGACAACATTAT ACATGGCTCTGAAATATCTGACACAATGTAAAC ATTGCAGGCACCTGCATTTTATGTTTTTTTTTCA ACAAATGTGACTAATTTGAAACTTTTATGAACTT CTGAGCTGTCCCCTTGCAATTCAACCGCAGTTTG AATTAATCATATCAAATCAGTTTTAATTTTTTAA ATTGTACTTCAGAGTCTATATTTCAAGGGCACAT TTTCTCACTACTATTTTAATACATTAAAGGACTA AATAATCTTTCAGAGATGCTGGAAACAAATCAT TTGCTTTATATGTTTCATTAGAATACCAATGAAA CATACAACTTGAAAATTAGTAATAGTATTTTTGA AGATCCCATTTCTAATTGGAGATCTCTTTAATTT CGATCAACTTATAATGTGTAGTACTATATTAAGT GCACTTGAGTGGAATTCAACATTTGACTAATAA AATGAGTTCATCATGTTGGCAAGTGATGTGGCA ATTATCTCTGGTGACAAAAGAGTAAAATCAAAT ATTTCTGCCTGTTACAAATATCAAGGAAGACCTG CTACTATGAAATAGATGACATTAATCTGTCTTCA CTGTTTATAATACGGATGGATTTTTTTTCAAATC AGTGTGTGTTTTGAGGTCTTATGTAATTGATGAC ATTTGAGAGAAATGGTGGCTTTTTTTAGCTACCT CTTTGTTCATTTAAGCACCAGTAAAGATCATGTC TTTTTATAGAAGTGTAGATTTTCTTTGTGACTTTG CTATCGTGCCTAAAGCTCTAAATATAGGTGAATG TGTGATGAATACTCAGATTATTTGTCTCTCTATA	15

		TAATTAGTTTGGTACTAAGTTTCTCAAAAAATTA TTAACACATGAAAGACAATCTCTAAACCAGAAA AAGAAGTAGTACAAATTTTGTTACTGTAATGCTC GCGTTTAGTGAGTTTAAAACACACAGTATCTTTT GGTTTTATAATCAGTTTCTATTTTGCTGTGCCTGA GATTAAGATCTGTGTATGTGTGTGTGTGTGTGTG TGCGTTTGTGTGTTAAAGCAGAAAAGACTTTTTT AAAAGTTTTAAGTGATAAATGCAATTTGTTAATT GATCTTAGATCACTAGTAAACTCAGGGCTGAATT ATACCATGTATATTCTATTAGAAGAAAGTAAAC ACCATCTTTATTCCTGCCCTTTTTCTTCTCTCAAA GTAGTTGTAGTTATATCTAGAAAGAAGCAATTTT GATTTCTTGAAAAGGTAGTTCCTGCACTCAGTTT AAACTAAAAATAATCATACTTGGATTTTATTTAT TTTTGTCATAGTAAAAATTTTAATTTATATATATT TTTATTTAGTATTATCTTATTCTTTGCTATTTGCC AATCCTTTGTCATCAATTGTGTTAAATGAATTGA AAATTCATGCCCTGTTCATTTTATTTTACTTTATT GGTTAGGATATTTAAAGGATTTTTGTATATATAA TTTCTTAAATTAATATTCCAAAAGGTTAGTGGAC TTAGATTATAAATTATGGCAAAAATCTAAAAAC AACAAAAATGATTTTTATACATTCTATTTCATTA TTCCTCTTTTTCCAATAAGTCATACAATTGGTAG ATATGACTTATTTTATTTTTGTATTATTCACTATA TCTTTATGATATTTAAGTATAAATAATTAAAAAA ATTTATTGTACCTTATAGTCTGTCACCAAAAAAA AAAAATTATCTGTAGGTAGTGAAATGCTAATGTT GATTTGTCTTTAAGGGCTTGTTAACTATCCTTTAT TTTCTCATTTGTCTTAAATTAGGAGTTTGTGTTTA AATTACTCATCTAAGCAAAAAATGTATATAAAT CCCATTACTGGGTATATACCCAAAGGATTATAA ATCATGCTGCTATAAAGACACATGCACACGTAT GTTTATTGCAGCACTATTCACAATAGCAAAGACT TGGAACCAACCCAAATGTCCATCAATGATAGAC TTGATTAAGAAAATGTGCACATATACACCATGG AATACTATGCAGCCATAAAAAAGGATGAGTTCA TGTCCTTTGTAGGGACATGGATAAAGCTGGAAA CCATCATTCTGAGCAAACTATTGCAAGGACAGA AAACCAAACACTGCATGTTCTCACTCATAGGTG GGAATTGAACAATGAGAACACTTGGACACAAGG TGGGGAACACCACACACCAGGGCCTGTCATGGG GTGGGGGGAGTGGGGAGGGATAGCATTAGGAG ATATACCTAATGTAAATGATGAGTTAATGGGTG CAGCACACCAACATGGCACATGTATACATATGT AGCAAACCTGCACGTTGTGCACATGTACCCTAG AACTTAAAGTATAATTAAAAAAAAAAAGAAAAC AGAAGCTATTTATAAAGAAGTTATTTGCTGAAAT AAATGTGATCTTTCCCATTAAAAAAATAAAGAA ATTTTGGGGTAAAAAAACACAATATATTGTATTC TTGAAAAATTCTAAGAGAGTGGATGTGAAGTGT TCTCACCACAAAAGTGATAACTAATTGAGGTAA	

		TGCACATATTAATTAGAAAGATTTTGTCATTCCA CAATGTATATATACTTAAAAATATGTTATACACA ATAAATACATACATTAAAAAATAAGTAAATGTA	
3UTR-012	Col6al; collagen, type VI, alpha 1	CCCACCCTGCACGCCGGCACCAAACCCTGTCCTC CCACCCCTCCCCACTCATCACTAAACAGAGTAA AATGTGATGCGAATTTTCCCGACCAACCTGATTC GCTAGATTTTTTTTAAGGAAAAGCTTGGAAAGCC AGGACACAACGCTGCTGCCTGCTTTGTGCAGGG TCCTCCGGGGCTCAGCCCTGAGTTGGCATCACCT GCGCAGGGCCCTCTGGGGCTCAGCCCTGAGCTA GTGTCACCTGCACAGGGCCCTCTGAGGCTCAGC CCTGAGCTGGCGTCACCTGTGCAGGGCCCTCTGG GGCTCAGCCCTGAGCTGGCCTCACCTGGGTTCCC CACCCCGGGCTCTCCTGCCCTGCCCTCCTGCCCG CCCTCCCTCCTGCCTGCGCAGCTCCTTCCCTAGG CACCTCTGTGCTGCATCCCACCAGCCTGAGCAAG ACGCCCTCTCGGGGCCTGTGCCGCACTAGCCTCC CTCTCCTCTGTCCCCATAGCTGGTTTTTCCCACCA ATCCTCACCTAACAGTTACTTTACAATTAAACTC AAAGCAAGCTCTTCTCCTCAGCTTGGGGCAGCC ATTGGCCTCTGTCTCGTTTTGGGAAACCAAGGTC AGGAGGCCGTTGCAGACATAAATCTCGGCGACT CGGCCCCGTCTCCTGAGGGTCCTGCTGGTGACCG GCCTGGACCTTGGCCCTACAGCCCTGGAGGCCG CTGCTGACCAGCACTGACCCCGACCTCAGAGAG TACTCGCAGGGGCGCTGGCTGCACTCAAGACCC TCGAGATTAACGGTGCTAACCCCGTCTGCTCCTC CCTCCCGCAGAGACTGGGGCCTGGACTGGACAT GAGAGCCCCTTGGTGCCACAGAGGGCTGTGTCT TACTAGAAACAACGCAAACCTCTCCTTCCTCAGA ATAGTGATGTGTTCGACGTTTTATCAAAGGCCCC CTTTCTATGTTCATGTTAGTTTTGCTCCTTCTGTG TTTTTTTCTGAACCATATCCATGTTGCTGACTTTT CCAAATAAAGGTTTTCACTCCTCTC	16
3UTR-013	Calr; calreticulin	AGAGGCCTGCCTCCAGGGCTGGACTGAGGCCTG AGCGCTCCTGCCGCAGAGCTGGCCGCGCCAAAT AATGTCTCTGTGAGACTCGAGAACTTTCATTTTT TTCCAGGCTGGTTCGGATTTGGGGTGGATTTTGG TTTTGTTCCCCTCCTCCACTCTCCCCCACCCCCTC CCCGCCCTTTTTTTTTTTTTTTTTTAAACTGGTAT TTTATCTTTGATTCTCCTTCAGCCCTCACCCCTGG TTCTCATCTTTCTTGATCAACATCTTTTCTTGCCT CTGTCCCCTTCTCTCATCTCTTAGCTCCCCTCCAA CCTGGGGGGCAGTGGTGTGGAGAAGCCACAGGC CTGAGATTTCATCTGCTCTCCTTCCTGGAGCCCA GAGGAGGGCAGCAGAAGGGGGTGGTGTCTCCAA CCCCCCAGCACTGAGGAAGAACGGGGCTCTTCT CATTTCACCCCTCCCTTTCTCCCCTGCCCCCAGG ACTGGGCCACTTCTGGGTGGGGCAGTGGGTCCC AGATTGGCTCACACTGAGAATGTAAGAACTACA AACAAAATTTCTATTAAATTAAATTTTGTGTCTC	17

		C	
3UTR-014	Collal; collagen, type I, alpha 1	CTCCCTCCATCCCAACCTGGCTCCCTCCCACCCA ACCAACTTTCCCCCCAACCCGGAAACAGACAAG CAACCCAAACTGAACCCCCTCAAAAGCCAAAAA ATGGGAGACAATTTCACATGGACTTTGGAAAAT ATTTTTTTCCTTTGCATTCATCTCTCAAACTTAGT TTTTATCTTTGACCAACCGAACATGACCAAAAAC CAAAAGTGCATTCAACCTTACCAAAAAAAAAAA AAAAAAAAGAATAAATAAATAACTTTTTAAAAA AGGAAGCTTGGTCCACTTGCTTGAAGACCCATG CGGGGGTAAGTCCCTTTCTGCCCGTTGGGCTTAT GAAACCCCAATGCTGCCCTTTCTGCTCCTTTCTC CACACCCCCCTTGGGGCCTCCCCTCCACTCCTTC CCAAATCTGTCTCCCCAGAAGACACAGGAAACA ATGTATTGTCTGCCCAGCAATCAAAGGCAATGCT CAAACACCCAAGTGGCCCCCACCCTCAGCCCGC TCCTGCCCGCCCAGCACCCCCAGGCCCTGGGGG ACCTGGGGTTCTCAGACTGCCAAAGAAGCCTTG CCATCTGGCGCTCCCATGGCTCTTGCAACATCTC CCCTTCGTTTTTGAGGGGGTCATGCCGGGGGAGC CACCAGCCCCTCACTGGGTTCGGAGGAGAGTCA GGAAGGGCCACGACAAAGCAGAAACATCGGATT TGGGGAACGCGTGTCAATCCCTTGTGCCGCAGG GCTGGGCGGGAGAGACTGTTCTGTTCCTTGTGTA ACTGTGTTGCTGAAAGACTACCTCGTTCTTGTCT TGATGTGTCACCGGGGCAACTGCCTGGGGGCGG GGATGGGGGCAGGGTGGAAGCGGCTCCCCATTT TATACCAAAGGTGCTACATCTATGTGATGGGTG GGGTGGGGAGGGAATCACTGGTGCTATAGAAAT TGAGATGCCCCCCCAGGCCAGCAAATGTTCCTTT TTGTTCAAAGTCTATTTTTATTCCTTGATATTTTT CTTTTTTTTTTTTTTTTTTTGTGGATGGGGACTTG TGAATTTTTCTAAAGGTGCTATTTAACATGGGAG GAGAGCGTGTGCGGCTCCAGCCCAGCCCGCTGC TCACTTTCCACCCTCTCTCCACCTGCCTCTGGCTT CTCAGGCCTCTGCTCTCCGACCTCTCTCCTCTGA AACCCTCCTCCACAGCTGCAGCCCATCCTCCCGG CTCCCTCCTAGTCTGTCCTGCGTCCTCTGTCCCCG GGTTTCAGAGACAACTTCCCAAAGCACAAAGCA GTTTTTCCCCCTAGGGGTGGGAGGAAGCAAAAG ACTCTGTACCTATTTTGTATGTGTATAATAATTT GAGATGTTTTTAATTATTTTGATTGCTGGAATAA AGCATGTGGAAATGACCCAAACATAATCCGCAG TGGCCTCCTAATTTCCTTCTTTGGAGTTGGGGGA GGGGTAGACATGGGGAAGGGGCTTTGGGGTGAT GGGCTTGCCTTCCATTCCTGCCCTTTCCCTCCCCA CTATTCTCTTCTAGATCCCTCCATAACCCCACTC CCCTTTCTCTCACCCTTCTTATACCGCAAACCTTT CTACTTCCTCTTTCATTTTCTATTCTTGCAATTTC CTTGCACCTTTTCCAAATCCTCTTCTCCCCTGCAA TACCATACAGGCAATCCACGTGCACAACACACA	18

		CACACACTCTTCACATCTGGGGTTGTCCAAACCT CATACCCACTCCCCTTCAAGCCCATCCACTCTCC ACCCCCTGGATGCCCTGCACTTGGTGGCGGTGG GATGCTCATGGATACTGGGAGGGTGAGGGGAGT GGAACCCGTGAGGAGGACCTGGGGGCCTCTCCT TGAACTGACATGAAGGGTCATCTGGCCTCTGCTC CCTTCTCACCCACGCTGACCTCCTGCCGAAGGAG CAACGCAACAGGAGAGGGGTCTGCTGAGCCTGG CGAGGGTCTGGGAGGGACCAGGAGGAAGGCGT GCTCCCTGCTCGCTGTCCTGGCCCTGGGGGAGTG AGGGAGACAGACACCTGGGAGAGCTGTGGGGA AGGCACTCGCACCGTGCTCTTGGGAAGGAAGGA GACCTGGCCCTGCTCACCACGGACTGGGTGCCTC GACCTCCTGAATCCCCAGAACACAACCCCCCTG GGCTGGGGTGGTCTGGGGAACCATCGTGCCCCC GCCTCCCGCCTACTCCTTTTTAAGCTT	
3UTR-015	Plodl; procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1	TTGGCCAGGCCTGACCCTCTTGGACCTTTCTTCT TTGCCGACAACCACTGCCCAGCAGCCTCTGGGA CCTCGGGGTCCCAGGGAACCCAGTCCAGCCTCC TGGCTGTTGACTTCCCATTGCTCTTGGAGCCACC AATCAAAGAGATTCAAAGAGATTCCTGCAGGCC AGAGGCGGAACACACCTTTATGGCTGGGGCTCT CCGTGGTGTTCTGGACCCAGCCCCTGGAGACAC CATTCACTTTTACTGCTTTGTAGTGACTCGTGCTC TCCAACCTGTCTTCCTGAAAAACCAAGGCCCCCT TCCCCCACCTCTTCCATGGGGTGAGACTTGAGCA GAACAGGGGCTTCCCCAAGTTGCCCAGAAAGAC TGTCTGGGTGAGAAGCCATGGCCAGAGCTTCTC CCAGGCACAGGTGTTGCACCAGGGACTTCTGCTT CAAGTTTTGGGGTAAAGACACCTGGATCAGACT CCAAGGGCTGCCCTGAGTCTGGGACTTCTGCCTC CATGGCTGGTCATGAGAGCAAACCGTAGTCCCC TGGAGACAGCGACTCCAGAGAACCTCTTGGGAG ACAGAAGAGGCATCTGTGCACAGCTCGATCTTC TACTTGCCTGTGGGGAGGGGAGTGACAGGTCCA CACACCACACTGGGTCACCCTGTCCTGGATGCCT CTGAAGAGAGGGACAGACCGTCAGAAACTGGA GAGTTTCTATTAAAGGTCATTTAAACCA	19
3UTR-016	Nucbl; nucleobindin 1	TCCTCCGGGACCCCAGCCCTCAGGATTCCTGATG CTCCAAGGCGACTGATGGGCGCTGGATGAAGTG GCACAGTCAGCTTCCCTGGGGGCTGGTGTCATGT TGGGCTCCTGGGGCGGGGGCACGGCCTGGCATT TCACGCATTGCTGCCACCCCAGGTCCACCTGTCT CCACTTTCACAGCCTCCAAGTCTGTGGCTCTTCC CTTCTGTCCTCCGAGGGGCTTGCCTTCTCTCGTG TCCAGTGAGGTGCTCAGTGATCGGCTTAACTTAG AGAAGCCCGCCCCCTCCCCTTCTCCGTCTGTCCC AAGAGGGTCTGCTCTGAGCCTGCGTTCCTAGGTG GCTCGGCCTCAGCTGCCTGGGTTGTGGCCGCCCT AGCATCCTGTATGCCCACAGCTACTGGAATCCCC GCTGCTGCTCCGGGCCAAGCTTCTGGTTGATTAA	20

		TGAGGGCATGGGGTGGTCCCTCAAGACCTTCCC CTACCTTTTGTGGAACCAGTGATGCCTCAAAGAC AGTGTCCCCTCCACAGCTGGGTGCCAGGGGCAG GGGATCCTCAGTATAGCCGGTGAACCCTGATAC CAGGAGCCTGGGCCTCCCTGAACCCCTGGCTTCC AGCCATCTCATCGCCAGCCTCCTCCTGGACCTCT TGGCCCCCAGCCCCTTCCCCACACAGCCCCAGA AGGGTCCCAGAGCTGACCCCACTCCAGGACCTA GGCCCAGCCCCTCAGCCTCATCTGGAGCCCCTGA AGACCAGTCCCACCCACCTTTCTGGCCTCATCTG ACACTGCTCCGCATCCTGCTGTGTGTCCTGTTCC ATGTTCCGGTTCCATCCAAATACACTTTCTGGAA CAAA	
3UTR-017	α-globin	GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTT GGGCCTCCCCCCAGCCCCTCCTCCCCTTCCTGCA CCCGTACCCCCGTGGTCTTTGAATAAAGTCTGAG TGGGCGGC	21

[00261] It should be understood that those listed in the previous tables are examples and that any UTR from any gene may be incorporated into the respective first or second flanking region of the primary construct. Furthermore, multiple wild-type UTRs of any known gene may be utilized. It is also within the scope of the present invention to provide artificial UTRs which are not variants of wild type genes. These UTRs or portions thereof may be placed in the same orientation as in the transcript from which they were selected or may be altered in orientation or location. Hence a 5' or 3' UTR may be inverted, shortened, lengthened, made chimeric with one or more other 5' UTRs or 3' UTRs. As used herein, the term "altered" as it relates to a UTR sequence, means that the UTR has been changed in some way in relation to a reference sequence. For example, a 3' or 5' UTR may be altered relative to a wild type or native UTR by the change in orientation or location as taught above or may be altered by the inclusion of additional nucleotides, deletion of nucleotides, swapping or transposition of nucleotides. Any of these changes producing an "altered" UTR (whether 3' or 5') comprise a variant UTR.

[00262] In one embodiment, a double, triple or quadruple UTR such as a 5' or 3' UTR may be used. As used herein, a "double" UTR is one in which two copies of the same UTR are encoded either in series or substantially in series. For example, a double beta-globin 3' UTR may be used as described in US Patent publication 20100129877, the contents of which are incorporated herein by reference in its entirety.

[00263] It is also within the scope of the present invention to have patterned UTRs. As used herein "patterned UTRs" are those UTRs which reflect a repeating or alternating pattern, such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than 3 times. In these patterns, each letter, A, B, or C represent a different UTR at the nucleotide level.

[00264] In one embodiment, flanking regions are selected from a family of transcripts whose proteins share a common function, structure, feature of property. For example, oncology-related polypeptides of interest may belong to a family of proteins which are expressed in a particular cell, tissue or at some time during development. The UTRs from any of these genes may be swapped for any other UTR of the same or different family of proteins to create a new chimeric primary transcript. As used herein, a "family of proteins" is used in the broadest sense to refer to a group of two or more oncology-related polypeptides of interest which share at least one function, structure, feature, localization, origin, or expression pattern.

[00265] After optimization (if desired), the signal-sensor primary construct components are reconstituted and transformed into a vector such as, but not limited to, plasmids, viruses, cosmids, and artificial chromosomes. For example, the optimized construct may be reconstituted and transformed into chemically competent *E. coli,* yeast, neurospora, maize, drosophila, etc. where high copy plasmid-like or chromosome structures occur by methods described herein.

Stop Codons

[00266] In one embodiment, the signal-sensor primary constructs of the present invention may include at least two stop codons before the 3' untranslated region (UTR). The stop codon may be selected from TGA, TAA and TAG. In one embodiment, the signal-sensor primary constructs of the present invention include the stop codon TGA and one additional stop codon. In a further embodiment the addition stop codon may be TAA.

Vector Amplification

[00267] The vector containing the signal-sensor primary construct is then amplified and the plasmid isolated and purified using methods known in the art such as, but not limited to, a maxi prep using the Invitrogen PURELINK™ HiPure Maxiprep Kit (Carlsbad, CA).

Plasmid Linearization

[00268] The plasmid may then be linearized using methods known in the art such as, but not limited to, the use of restriction enzymes and buffers. The linearization reaction may be purified using methods including, for example Invitrogen's PURELINK™ PCR Micro Kit (Carlsbad, CA), and HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC) and Invitrogen's standard PURELINK™ PCR Kit (Carlsbad, CA). The purification method may be modified depending on the size of the linearization reaction which was conducted. The linearized plasmid is then used to generate cDNA for *in vitro* transcription (IVT) reactions.

cDNA Template Synthesis

[00269] A cDNA template may be synthesized by having a linearized plasmid undergo polymerase chain reaction (PCR). Table 4 is a listing of primers and probes that may be useful in the PCR reactions of the present invention. It should be understood that the listing is not exhaustive and that primer-probe design for any amplification is within the skill of those in the art. Probes may also contain chemically modified bases to increase base-pairing fidelity to the target molecule and base-pairing strength. Such modifications may include 5-methyl-Cytidine, 2, 6-di-amino-purine, 2'-fluoro, phosphoro-thioate, or locked nucleic acids.

Table 4. Primers and Probes

Primer/ Probe Identifier	Sequence (5′-3′)	Hybridization target	SEQ ID NO.
UFP	TTGGACCCTCGTACAGAAGCTAA TACG	cDNA Template	22
URP	T_{x160}CTTCCTACTCAGGCTTTATTC AAAGACCA	cDNA Template	23
GBA1	CCTTGACCTTCTGGAACTTC	Acid glucocerebrosidase	24
GBA2	CCAAGCACTGAAACGGATAT	Acid glucocerebrosidase	25
LUC1	GATGAAAAGTGCTCCAAGGA	Luciferase	26
LUC2	AACCGTGATGAAAAGGTACC	Luciferase	27
LUC3	TCATGCAGATTGGAAAGGTC	Luciferase	28

GCSF1	CTTCTTGGACTGTCCAGAGG	G-CSF	29
GCSF2	GCAGTCCCTGATACAAGAAC	G-CSF	30
GCSF3	GATTGAAGGTGGCTCGCTAC	G-CSF	31

[00270] *UFP is universal forward primer; URP is universal reverse primer.

[00271] In one embodiment, the cDNA may be submitted for sequencing analysis before undergoing transcription.

Signal-sensor polynucleotide Production (signal-sensor mRNA)

[00272] The process of signal-sensor polynucleotide production may include, but is not limited to, *in vitro* transcription, cDNA template removal and RNA clean-up, and capping and/or tailing reactions.

In Vitro Transcription

[00273] The cDNA produced in the previous step may be transcribed using an *in vitro* transcription (IVT) system. The system typically comprises a transcription buffer, nucleotide triphosphates (NTPs), an RNase inhibitor and a polymerase. The NTPs may be manufactured in house, may be selected from a supplier, or may be synthesized as described herein. The NTPs may be selected from, but are not limited to, those described herein including natural and unnatural (modified) NTPs. The polymerase may be selected from, but is not limited to, T7 RNA polymerase, T3 RNA polymerase and mutant polymerases such as, but not limited to, polymerases able to be incorporated into modified nucleic acids.

RNA Polymerases

[00274] Any number of RNA polymerases or variants may be used in the design of the signal-sensor primary constructs of the present invention.

[00275] RNA polymerases may be modified by inserting or deleting amino acids of the RNA polymerase sequence. As a non-limiting example, the RNA polymerase may be modified to exhibit an increased ability to incorporate a 2'-modified nucleotide triphosphate compared to an unmodified RNA polymerase (see International Publication WO2008078180 and U.S. Patent 8,101,385; herein incorporated by reference in their entireties).

[00276] Variants may be obtained by evolving an RNA polymerase, optimizing the RNA polymerase amino acid and/or nucleic acid sequence and/or by using other methods known in the art. As a non-limiting example, T7 RNA polymerase variants may be evolved using the continuous directed evolution system set out by Esvelt *et al.* (Nature (201 1) 472(7344):499-503; herein incorporated by reference in its entirety) where clones of T7 RNA polymerase may encode at least one mutation such as, but not limited to, lysine at position 93 substituted for threonine (K93T), I4M, A7T, E63V, V64D, A65E, D66Y, T76N, C125R, S128R, A136T, N165S, G175R, H176L, Y178H, F182L, L196F, G198V, D208Y, E222K, S228A, Q239R, T243N, G259D, M267I, G280C, H300R, D351A, A354S, E356D, L360P, A383V, Y385C, D388Y, S397R, M401T, N410S, K450R, P451T, G452V, E484A, H523L, H524N, G542V, E565K, K577E, K577M, N601S, S684Y, L699I, K713E, N748D, Q754R, E775K, A827V, D851N or L864F. As another non-limiting example, T7 RNA polymerase variants may encode at least mutation as described in U.S. Pub. Nos. 20100120024 and 200701 171 12; herein incorporated by reference in their entireties. Variants of RNA polymerase may also include, but are not limited to, substitutional variants, conservative amino acid substitution, insertional variants, deletional variants and/or covalent derivatives.

[00277] In one embodiment, the signal-sensor primary construct may be designed to be recognized by the wild type or variant RNA polymerases. In doing so, the signal-sensor primary construct may be modified to contain sites or regions of sequence changes from the wild type or parent primary construct.

[00278] In one embodiment, the signal-sensor primary construct may be designed to include at least one substitution and/or insertion upstream of an RNA polymerase binding or recognition site, downstream of the RNA polymerase binding or recognition site, upstream of the TATA box sequence, downstream of the TATA box sequence of the signal-sensor primary construct but upstream of the coding region of the primary construct, within the 5'UTR, before the 5'UTR and/or after the 5'UTR.

[00279] In one embodiment, the 5'UTR of the signal-sensor primary construct may be replaced by the insertion of at least one region and/or string of nucleotides of the same base. The region and/or string of nucleotides may include, but is not limited to, at least 3, at least 4, at least 5, at least 6, at least 7 or at least 8 nucleotides and the nucleotides may

be natural and/or unnatural. As a non-limiting example, the group of nucleotides may include 5-8 adenine, cytosine, thymine, a string of any of the other nucleotides disclosed herein and/or combinations thereof.

[00280] In one embodiment, the 5'UTR of the signal-sensor primary construct may be replaced by the insertion of at least two regions and/or strings of nucleotides of two different bases such as, but not limited to, adenine, cytosine, thymine, any of the other nucleotides disclosed herein and/or combinations thereof. For example, the 5'UTR may be replaced by inserting 5-8 adenine bases followed by the insertion of 5-8 cytosine bases. In another example, the 5'UTR may be replaced by inserting 5-8 cytosine bases followed by the insertion of 5-8 adenine bases.

[00281] In one embodiment, the signal-sensor primary construct may include at least one substitution and/or insertion downstream of the transcription start site which may be recognized by an RNA polymerase. As a non-limiting example, at least one substitution and/or insertion may occur downstream the transcription start site by substituting at least one nucleic acid in the region just downstream of the transcription start site (such as, but not limited to, +1 to +6). Changes to region of nucleotides just downstream of the transcription start site may affect initiation rates, increase apparent nucleotide triphosphate (NTP) reaction constant values, and increase the dissociation of short transcripts from the transcription complex curing initial transcription (Brieba et al, Biochemistry (2002) 41: 5144-5149; herein incorporated by reference in its entirety). The modification, substitution and/or insertion of at least one nucleic acid may cause a silent mutation of the nucleic acid sequence or may cause a mutation in the amino acid sequence.

[00282] In one embodiment, the signal-sensor primary construct may include the substitution of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12 or at least 13 guanine bases downstream of the transcription start site.

[00283] In one embodiment, the signal-sensor primary construct may include the substitution of at least 1, at least 2, at least 3, at least 4, at least 5 or at least 6 guanine bases in the region just downstream of the transcription start site. As a non-limiting example, if the nucleotides in the region are GGGAGA the guanine bases may be

substituted by at least 1, at least 2, at least 3 or at least 4 adenine nucleotides. In another non-limiting example, if the nucleotides in the region are GGGAGA the guanine bases may be substituted by at least 1, at least 2, at least 3 or at least 4 cytosine bases. In another non-limiting example, if the nucleotides in the region are GGGAGA the guanine bases may be substituted by at least 1, at least 2, at least 3 or at least 4 thymine, and/or any of the nucleotides described herein.

[00284] In one embodiment, the signal-sensor primary construct may include at least one substitution and/or insertion upstream of the start codon. For the purpose of clarity, one of skill in the art would appreciate that the start codon is the first codon of the protein coding region whereas the transcription start site is the site where transcription begins. The signal-sensor primary construct may include, but is not limited to, at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7 or at least 8 substitutions and/or insertions of nucleotide bases. The nucleotide bases may be inserted or substituted at 1, at least 1, at least 2, at least 3, at least 4 or at least 5 locations upstream of the start codon. The nucleotides inserted and/or substituted may be the same base (e.g., all A or all C or all T or all G), two different bases (e.g., A and C, A and T, or C and T), three different bases (e.g., A, C and T or A, C and T) or at least four different bases. As a non-limiting example, the guanine base upstream of the coding region in the signal-sensor primary construct may be substituted with adenine, cytosine, thymine, or any of the nucleotides described herein. In another non-limiting example the substitution of guanine bases in the signal-sensor primary construct may be designed so as to leave one guanine base in the region downstream of the transcription start site and before the start codon (see Esvelt *et al.* Nature (201 1) 472(7344):499-503; herein incorporated by reference in its entirety). As a non-limiting example, at least 5 nucleotides may be inserted at 1 location downstream of the transcription start site but upstream of the start codon and the at least 5 nucleotides may be the same base type.

cDNA Template Removal and Clean-Up

[00285] The cDNA template may be removed using methods known in the art such as, but not limited to, treatment with Deoxyribonuclease I (DNase I). RNA clean-up may also include a purification method such as, but not limited to, AGENCOURT® CLEANSEQ® system from Beckman Coulter (Danvers, MA), HPLC based purification

methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC) .

Capping and/or Tailing Reactions

[00286] The signal-sensor primary construct or mmRNA may also undergo capping and/or tailing reactions. A capping reaction may be performed by methods known in the art to add a 5' cap to the 5' end of the signal-sensor primary construct. Methods for capping include, but are not limited to, using a Vaccinia Capping enzyme (New England Biolabs, Ipswich, MA).

[00287] A poly-A tailing reaction may be performed by methods known in the art, such as, but not limited to, 2' O-methyltransferase and by methods as described herein. If the signal-sensor primary construct generated from cDNA does not include a poly-T, it may be beneficial to perform the poly-A-tailing reaction before the signal-sensor primary construct is cleaned.

Purification

[00288] Signal-sensor primary construct or mmRNA purification may include, but is not limited to, mRNA or mmRNA clean-up, quality assurance and quality control. mRNA or mmRNA clean-up may be performed by methods known in the arts such as, but not limited to, AGENCOURT® beads (Beckman Coulter Genomics, Danvers, MA), poly-T beads, LNA™ oligo-T capture probes (EXIQON® Inc, Vedbaek, Denmark) or HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC). The term "purified" when used in relation to a polynucleotide such as a "purified mRNA or signal-sensor mmRNA" refers to one that is separated from at least one contaminant. As used herein, a "contaminant" is any substance which makes another unfit, impure or inferior. Thus, a purified signal-sensor polynucleotide (e.g., DNA and RNA) is present in a form or setting different from that in which it is found in nature, or a form or setting different from that which existed prior to subjecting it to a treatment or purification method.

[00289] A quality assurance and/or quality control check may be conducted using methods such as, but not limited to, gel electrophoresis, UV absorbance, or analytical HPLC.

[00290] In another embodiment, the signal-sensor mRNA or mmRNA may be sequenced by methods including, but not limited to reverse-transcriptase-PCR.

[00291] In one embodiment, the signal-sensor mRNA or mmRNA may be quantified using methods such as, but not limited to, ultraviolet visible spectroscopy (UV/Vis). A non-limiting example of a UV/Vis spectrometer is a NANODROP® spectrometer (ThermoFisher, Waltham, MA). The quantified signal-sensor mRNA or mmRNA may be analyzed in order to determine if the signal-sensor mRNA or mmRNA may be of proper size, check that no degradation of the signal-sensor mRNA or mmRNA has occurred. Degradation of the signal-sensor mRNA and/or mmRNA may be checked by methods such as, but not limited to, agarose gel electrophoresis, HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC), liquid chromatography-mass spectrometry (LCMS), capillary electrophoresis (CE) and capillary gel electrophoresis (CGE).

Signal Peptides or Proteins

[00292] The signal-sensor primary constructs or mmRNA may also encode additional features which facilitate trafficking of the polypeptides to therapeutically relevant sites. One such feature which aids in protein trafficking is the signal peptide sequence. As used herein, a "signal sequence" or "signal peptide" is a polynucleotide or polypeptide, respectively, which is from about 9 to 200 nucleotides (3-60 amino acids) in length which is incorporated at the 5' (or N-terminus) of the coding region or polypeptide encoded, respectively. Addition of these sequences result in trafficking of the encoded oncology-related polypeptide to the endoplasmic reticulum through one or more secretory pathways. Some signal peptides are cleaved from the protein by signal peptidase after the proteins are transported.

[00293] Table 5 is a representative listing of signal proteins or peptides which may be incorporated for encoding by the signal-sensor polynucleotides, primary constructs or mmRNA of the invention.

Table 5. Signal Peptides

ID	Description	NUCLEOTIDE SEQUENCE (5′-3′)	SEQ ID NO.	ENCODED PEPTIDE	SEQ ID NO.
SS-001	α-1-antitrypsin	ATGATGCCATCCTCAGTCTCA TGGGGTATTTTGCTCTTGGCG GGTCTGTGCTGTCTCGTGCCG GTGTCGCTCGCA	32	MMPSSVS WGILLAGL CCLVPVSL A	94
SS-002	G-CSF	ATGGCCGGACCGGCGACTCAG TCGCCCATGAAACTCATGGCC CTGCAGTTGTTGCTTTGGCAC TCAGCCCTCTGGACCGTCCAA GAGGCG	33	MAGPATQ SPMKLMA LQLLLWH SALWTVQ EA	95
SS-003	Factor IX	ATGCAGAGAGTGAACATGATT ATGGCCGAGTCCCCATCGCTC ATCACAATCTGCCTGCTTGGT ACCTGCTTTCCGCCGAATGCA CTGTCTTTCTGGATCACGAGA ATGCGAATAAGATCTTGAACC GACCCAAACGG	34	MQRVNMI MAESPSLI TICLLGYL LSAECTVF LDHENAN KILNRPKR	96
SS-004	Prolactin	ATGAAAGGATCATTGCTGTTG CTCCTCGTGTCGAACCTTCTG CTTTGCCAGTCCGTAGCCCCC	35	MKGSLLL LLVSNLLL CQSVAP	97
SS-005	Albumin	ATGAAATGGGTGACGTTCATC TCACTGTTGTTTTTGTTCTCGT CCGCCTACTCCAGGGGAGTAT TCCGCCGA	36	MKWVTFI SLLFLFSS AYSRG VFRR	98
SS-006	HMMSP38	ATGTGGTGGCGGCTCTGGTGG CTGCTCCTGTTGCTCCTCTTGC TGTGGCCCATGGTGTGGGCA	37	MWWRLW WLLLLLLL LPMWA	99
MLS-001	ornithine carbamoyltransferase	TGCTCTTTAACCTCCGCATCCT GTTGAATAACGCTGCGTTCCG AAATGGGCATAACTTCATGGT ACGCAACTTCAGATGCGGCCA GCCACTCCAG	38	MLFNLRIL LNNAAFR NGHNFMV RNFRCGQP LQ	100
MLS-002	Cytochrome C Oxidase subunit 8A	ATGTCCGTCTTGACACCCCTG CTCTTGAGAGGGCTGACGGGG TCCGCTAGACGCCTGCCGGTA CCGCGAGCGAAGATCCACTCC CTG	39	MSVLTPLL LRGLTGSA RRLPVPRA KIHSL	101
MLS-003	Cytochrome C Oxidase subunit 8A	ATGAGCGTGCTCACTCCGTTG CTTCTTCGAGGGCTTACGGGA TCGGCTCGGAGGTTGCCCGTC CCGAGAGCGAAGATCCATTCG TTG	40	MSVLTPLL LRGLTGSA RRLPVPRA KIHSL	102
SS-007	Type III, bacterial	TGACAAAAATAACTTTATCTC CCCAGAATTTTAGAATCCAAA AACAGGAAACCACACTACTA AAAGAAAAATCAACCGAGAA AAATTCTTTAGCAAAAAGTAT	41	MVTKITLS PQNFRIQK QETTLLKE KSTEKNSL AKSILAVK	103

		TCTCGCAGTAAAAATCACTTC ATCGAATTAAGGTCAAAATTA TCGGAACGTTTTATTTCGCAT AAGAACACT		NHFIELRS KLSERFIS HKNT	
SS-008	Viral	ATGCTGAGCTTTGTGGATACC CGCACCCTGCTGCTGCTGGCG GTGACCAGCTGCCTGGCGACC TGCCAG	42	MLSFVDT RTLLLLAV TSCLATCQ	104
SS-009	viral	ATGGGCAGCAGCCAGGCGCC GCGCATGGGCAGCGTGGGCG GCCATGGCCTGATGGCGCTGC TGATGGCGGGCCTGATTCTGC CGGGCATTCTGGCG	43	MGSSQAP RMGSVGG HGLMALL MAGLILPG ILA	105
SS-010	Viral	ATGGCGGGCATTTTTTATTTTC TGTTTAGCTTTCTGTTTGGCAT TTGCGAT	44	MAGIFYFL FSFLFGICD	106
SS-01 1	Viral	ATGGAAAACCGCCTGCTGCGC GTGTTTCTGGTGTGGGCGGCG CTGACCATGGATGGCGCGAGC GCG	45	MENRLLR VFLVWAA LTMDGAS A	107
SS-012	Viral	ATGGCGCGCCAGGGCTGCTTT GGCAGCTATCAGGTGATTAGC CTGTTTACCTTTGCGATTGGC GTGAACCTGTGCCTGGGC	46	MARQGCF GSYQVISL FTFAIGVN LCLG	108
SS-013	*Bacillus*	ATGAGCCGCCTGCCGGTGCTG CTGCTGCTGCAGCTGCTGGTG CGCCCGGGCCTGCAG	47	MSRLPVLL LLQLLVRP GLQ	109
SS-014	*Bacillus*	ATGAAACAGCAGAAACGCCT GTATGCGCGCCTGCTGACCCT GCTGTTTGCGCTGATTTTTCTG CTGCCGCATAGCAGCGCGAGC GCG	48	MKQQKRL YARLLTLL FALIFLLPH SSASA	110
SS-015	Secretion signal	ATGGCGACGCCGCTGCCTCCG CCCTCCCCGCGGCACCTGCGG CTGCTGCGGCTGCTGCTCTCC GCCCTCGTCCTCGGC	49	MATPLPPP SPRHLRLL RLLLSG	111
SS-016	Secretion signal	ATGAAGGCTCCGGGTCGGCTC GTGCTCATCATCCTGTGCTCC GTGGTCTTCTCT	50	MKAPGRL VLIILCSVV FS	112
SS-017	Secretion signal	ATGCTTCAGCTTTGGAAACTT GTTCTCCTGTGCGGCGTGCTC ACT	5 1	MLQLWKL LCGVLT	113
SS-018	Secretion signal	ATGCTTTATCTCCAGGGTTGG AGCATGCCTGCTGTGGCA	52	MLYLQGW SMPAVA	114
SS-019	Secretion signal	ATGGATAACGTGCAGCCGAA AATAAAACATCGCCCCTTCTG CTTCAGTGTGAAAGGCCACGT GAAGATGCTGCGGCTGGATAT TATCAACTCACTGGTAACAAC AGTATTCATGCTCATCGTATC	53	MDNVQPK IKHRPFCF SVKGHVK MLRLDIIN SLVTTVFM LIVSVLALI	115

		TGTGTTGGCACTGATACCA		P	
SS-020	Secretion signal	ATGCCCTGCCTAGACCAACAG CTCACTGTTCATGCCCTACCCT GCCCTGCCCAGCCCTCCTCTC TGGCCTTCTGCCAAGTGGGGT TCTTAACAGCA	54	MPCLDQQ LTVHALPC PAQPSSLA FCQVGFLT A	116
SS-021	Secretion signal	ATGAAAACCTTGTTCAATCCA GCCCCTGCCATTGCTGACCTG GATCCCCAGTTCTACACCCTC TCAGATGTGTTCTGCTGCAAT GAAAGTGAGGCTGAGATTTTA ACTGGCCTCACGGTGGGCAGC GCTGCAGATGCT	55	MKTLFNP APAIADLD PQFYTLSD VFCCNESE AEILTGLT VGSAADA	117
SS-022	Secretion signal	ATGAAGCCTCTCCTTGTTGTG TTTGTCTTTCTTTTCCTTTGGG ATCCAGTGCTGGCA	56	MKPLLVV FVFLFLWD PVLA	118
SS-023	Secretion signal	ATGTCCTGTTCCCTAAAGTTT ACTTTGATTGTAATTTTTTTTT ACTGTTGGCTTTCATCCAGC	57	MSCSLKFT LIVIFFTCT LSSS	119
SS-024	Secretion signal	ATGGTTCTTACTAAACCTCTTC AAAGAAATGGCAGCATGATG AGCTTTGAAAATGTGAAAGAA AAGAGCAGAGAAGGAGGGCC CCATGCACACACACCCGAAGA AGAATTGTGTTTCGTGGTAAC ACACTACCCTCAGGTTCAGAC CACACTCAACCTGTTTTTCCAT ATATTCAAGGTTCTTACTCAA CCACTTTCCCTTCTGTGGGGT	58	MVLTKPL QRNGSMM SFENVKEK SREGGPHA HTPEEELC FVVTHTPQ VQTTLNLF FHIFKVLT QPLSLLW G	120
SS-025	Secretion signal	ATGGCCACCCCGCCATTCCGG CTGATAAGGAAGATGTTTTCC TTCAAGGTGAGCAGATGGATG GGGCTTGCCTGCTTCCGGTCC CTGGCGGCATCC	59	MATPPFRL IRKMFSFK VSRWMGL ACFRSLAA S	121
SS-026	Secretion signal	ATGAGCTTTTTCCAACTCCTG ATGAAAAGGAAGGAACTCAT TCCCTTGGTGGTGTTCATGAC TGTGGCGGCGGGTGGAGCCTC ATCT	60	MSFFQLL MKRKELIP LVVFMTV AAGGASS	122
SS-027	Secretion signal	ATGGTCTCAGCTCTGCGGGGA GCACCCCTGATCAGGGTGCAC TCAAGCCCTGTTTCTTCTCCTT CTGTGAGTGGACCACGGAGGC TGGTGAGCTGCCTGTCATCCC AAAGCTCAGCTCTGAGC	61	MVSALRG APLIRVHS SPVSSPSV SGPAALVS CLSSQSSA LS	123
SS-028	Secretion signal	ATGATGGGGTCCCCAGTGAGT CATCTGCTGGCCGGCTTCTGT GTGTGGGTCGTCTTGGGC	62	MMGSPVS HLLAGFC VWVVLG	124
SS-029	Secretion signal	ATGGCAAGCATGGCTGCCGTG CTCACCTGGGCTCTGGCTCTT	63	MASMAAV LTWALAL	125

		CTTTCAGCGTTTTCGGCCACC CAGGCA		LSAFSATQ A	
SS-030	Secretion signal	ATGGTGCTCATGTGGACCAGT GGTGACGCCTTCAAGACGGCC TACTTCCTGCTGAAGGGTGCC CCTCTGCAGTTCTCCGTGTGC GGCCTGCTGCAGGTGCTGGTG GACCTGGCCATCCTGGGGCAG GCCTACGCC	64	MVLMWTS GDAFKTA YFLLKGAP LQFSVCGL LQVLVDL AILGQATA	126
SS-031	Secretion signal	ATGGATTTTGTCGCTGGAGCC ATCGGAGGCGTCTGCGGTGTT GCTGTGGGCTACCCCCTGGAC ACGGTGAAGGTCAGGATCCA GACGGAGCCAAAGTACACAG GCATCTGGCACTGCGTCCGGG ATACGTATCACCGAGAGCGCG TGTGGG GCTTCTACCGGGGCCTCTCGC TGCCCGTGTGCACGGTGTCCC TGGTATCTTCC	65	MDFVAGA IGGVCGV AVGYPLD TVKVRIQT EPLYTGIW HCVRDTY HRERVWG FYRGLSLP VCTVSLVS S	127
SS-032	Secretion signal	ATGGAGAAGCCCCTCTTCCCA TTAGTGCCTTTGCATTGGTTTG GCTTTGGCTACACAGCACTGG TTGTTTCTGGTGGGATCGTTG GCTATGTAAAAACAGGCAGC GTGCCGTCCCTGGCTGCAGGG CTGCTCTTCGGCAGTCTAGCC	66	MEKPLFPL VPLHWFG FGYTALV VSGGIVGY VKTGSVPS LAAGLLFG SLA	128
SS-033	Secretion signal	ATGGGTCTGCTCCTTCCCCTG GCACTCTGCATCCTAGTCCTG TGC	67	MGLLLPL ALCILVLC	129
SS-034	Secretion signal	ATGGGGATCCAGACGAGCCCC GTCCTGCTGGCCTCCCTGGGG GTGGGGCTGGTCACTCTGCTC GGCCTGGCTGTGGGC	68	MGIQTSPV LLASLGVG LVTLLGLA VG	130
SS-035	Secretion signal	ATGTCGGACCTGCTACTACTG GGCCTGATTGGGGGCCTGACT CTCTTACTGCTGCTGACGCTG CTAGCCTTTGCC	69	MSDLLLL GLIGGLTL LLLLTLLA FA	131
SS-036	Secretion signal	ATGGAGACTGTGGTGATTGTT GCCATAGGTGTGCTGGCCACC ATGTTTCTGGCTTCGTTTGCAG CCTTGGTGCTGGTTTGCAGGC AG	70	METVVIV AIGVLATI FLASFAAL VLVCRQ	132
SS-037	Secretion signal	ATGCGCGGCTCTGTGGAGTGC ACCTGGGGTTGGGGGCACTGT GCCCCCAGCCCCCTGCTCCTT TGGACTCTACTTCTGTTTGCA GCCCCATTTGGCCTGCTGGGG	7 1	MAGSVEC TWGWGH CAPSPLLL WTLLLFA APFGLLG	133
SS-038	Secretion signal	ATGATGCCGTCCCGTACCAAC CTGGCTACTGGAATCCCCAGT	72	MMPSRTN LATGIPSS	134

		AGTAAAGTGAAATATTCAAGG CTCTCCAGCACAGACGATGGC TACATTGACCTTCAGTTTAAG AAAACCCCTCCTAAGATCCCT TATAAGGCCATCGCACTTGCC ACTGTGCTGTTTTTGATTGGC GCC		KVKYSRLS STDDGYID LQFKKTPP KIPYKAIA LATVLFLI GA	
SS-039	Secretion signal	ATGGCCCTGCCCCAGATGTGT GACGGGAGCCACTTGGCCTCC ACCCTCCGCTATTGCATGACA GTCAGCGGCACAGTGGTTCTG GTGGCCGGGACGCTCTGCTTC GCT	73	MALPQMC DGSHLAST LRYCMTV SGTVVLV AGTLCFA	135
SS-041	Vrg-6	TGAAAAAGTGGTTCGTTGCTG CCGGCATCGGCGCTGCCGGAC TCATGCTCTCCAGCGCCGCCA	74	MKKWFVA AGIGAGLL MLSSAA	136
SS-042	PhoA	ATGAAACAGAGCACCATTGCG CTGGCGCTGCTGCCGCTGCTG TTTACCCCGGTGACCAAAGCG	75	MKQSTIAL ALLPLLFT PVTKA	137
SS-043	OmpA	ATGAAAAAAACCGCGATTGC GATTGCGGTGGCGCTGGCGGG CTTTGCGACCGTGGCGCAGGC G	76	MKKTAIAI AVALAGF ATVAQA	138
SS-044	STI	ATGAAAAAACTGATGCTGGCG ATTTTTTTTAGCGTGCTGAGCT TTCCGAGCTTTAGCCAGAGC	77	MKKLMLA IFFSVLSFP SFSQS	139
SS-045	STII	ATGAAAAAAAACATTGCGTTT CTGCTGGCGAGCATGTTTGTG TTTAGCATTGCGACCAACGCG TATGCG	78	MKKNIAFL LASMFVFS IATNAYA	140
SS-046	Amylase	ATGTTTGCGAAACGCTTTAAA ACCAGCCTGCTGCCGCTGTTT GCGGGCTTTCTGCTGCTGTTTC ATCTGGTGCTGGCGGGCCCGG CGGCGGCGAGC	79	MFAKRFK TSLLPLFA GFLLLFHL VLAGPAA AS	141
SS-047	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCGCGGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	80	MRFPSIFT AVLFAASS ALA	142
SS-048	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCACCGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	81	MRFPSIFT TVLFAASS ALA	143
SS-049	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCAGCGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	82	MRFPSIFTS VLFAASSA LA	144
SS-050	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCCATGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	83	MRFPSIFT HVLFAASS ALA	145
SS-051	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCATTGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	84	MRFPSIFTI VLFAASSA LA	146

SS-052	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCTTTGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	85	MRFPSIFTF VLFAASSA LA	147
SS-053	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCGAAGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	86	MRFPSIFT EVLFAASS ALA	148
SS-054	Alpha Factor	ATGCGCTTTCCGAGCATTTTT ACCGGCGTGCTGTTTGCGGCG AGCAGCGCGCTGGCG	87	MRFPSIFT GVLFAASS ALA	149
SS-055	Endoglucan ase V	ATGCGTTCCTCCCCCCTCCTCC GCTCCGCCGTTGTGGCCGCCC TGCCGGTGTTGGCCCTTGCC	88	MRSSPLLR SAVVAAL PVLALA	150
SS-056	Secretion signal	ATGGGCGCGGCGGCCGTGCGC TGGCACTTGTGCGTGCTGCTG GCCCTGGGCACACGCGGGCG GCTG	89	MGAAAVR WHLCVLL ALGTRGR L	151
SS-057	Fungal	ATGAGGAGCTCCCTTGTGCTG TTCTTTGTCTCTGCGTGGACG GCCTTGGCCAG	90	MRSSLVLF FVSAWTA LA	152
SS-058	Fibronectin	ATGCTCAGGGGTCCGGGACCC GGGCGGCTGCTGCTGCTAGCA GTCCTGTGCCTGGGGACATCG GTGCGCTGCACCGAAACCGGG AAGAGCAAGAGG	9 1	MLRGPGP GRLLLLAV LCLGTSVR CTETGKSK R	153
SS-059	Fibronectin	ATGCTTAGGGGTCCGGGGCCC GGGCTGCTGCTGCTGGCCGTC CAGCTGGGGACAGCGGTGCCC TCCACG	92	MLRGPGP GLLLLAV QCLGTAV PSTGA	154
SS-060	Fibronectin	ATGCGCCGGGGGGCCCTGACC GGGCTGCTCCTGGTCCTGTGC CTGAGTGTTGTGCTACGTGCA GCCCCCTCTGCAACAAGCAAG AAGCGCAGG	93	MRRGALT GLLLVLCL SVVLRAAP SATSKKRR	155

[00294] In the table, SS is secretion signal and MLS is mitochondrial leader signal. The signal-sensor primary constructs or mmRNA of the present invention may be designed to encode any of the signal peptide sequences of SEQ ID NOs 94-155, or fragments or variants thereof. These sequences may be included at the beginning of the oncology-related polypeptide coding region, in the middle or at the terminus or alternatively into a flanking region. Further, any of the signal-sensor polynucleotide primary constructs of the present invention may also comprise one or more of the sequences defined by SEQ ID NOs 32-93. These may be in the first region or either flanking region.

[00295] Additional signal peptide sequences which may be utilized in the present invention include those taught in, for example, databases such as those found at http://www.signalpeptide.de/ or http://proline.bic.nus.edu.sg/spdb/. Those described in US Patents 8,124,379; 7,413,875 and 7,385,034 are also within the scope of the invention and the contents of each are incorporated herein by reference in their entirety.

[00296] In one embodiment, the signal-sensor polynucleotide, primary constructs or mmRNA may include a nucleic acid sequence encoding a nuclear localization signal (NLS) and/or a nuclear export signal (NES). In one aspect, a signal-sensor polynucleotide, primary constructs or mmRNA may include a nucleic acid sequence encoding a nuclear localization signal (NLS). The signal-sensor polynucleotide, primary construct or mmRNA encoding a NLS would be able to traffic an oncology related polypeptide into the nucleus and deliver a survival or death signal to the nuclear microenvironment. In another aspect, the signal-sensor polynucleotide, primary constructs or mmRNA may include a nucleic acid sequence encoding a nuclear export signal such as NES1 and/or NES2. As a nonlimiting example, the signal-sensor polynucleotide, primary constructs or mmRNA may encode a NES1, NES2 and a NLS signal and an oncology related polypeptide or a scambled sequence which is not translatable in order to interact with HIF1-alpha to alter the transcritome of the cancer cells.

Target Selection

[00297] According to the present invention, the signal-sensor primary constructs comprise at least a first region of linked nucleosides encoding at least one oncology-related polypeptide of interest. The oncology-related polypeptides of interest or "targets" or oncology-related proteins and oncology-related peptides of the present invention are listed in Table 6, Table 7 and Table 41. Oncology-related polypeptides may be divided into classes based on their function and area of cancer intervention. For example, the classes may include targets associated with (1) apoptosis or Survival signal imbalance (AS targets). These may be caspase dependent or caspase independent targets; (2) replicative potential or anti-senescence (CC/S targets); (3) metabolic stress including the involvement of acidosis or hypoxia (0_2 > 1%) (M targets); (4) immune response (I targets); and (5) DNA damage/protection (DDR targets).

[00298] Shown in Table 6, in addition to the name and description of the gene encoding the oncology-related polypeptide of interest are the ENSEMBL Transcript ID (ENST), the ENSEMBL Protein ID (ENSP), each present where applicable, and when available the optimized sequence ID (OPT. SEQ ID). The targets are also categorized by group where "AS" refers to targets involved in apoptotic signaling; "M" refers to targets involved in metabolic processes and "CC/S" refers to targets involved in cell cycle and senescense.

Table 6. Oncology Related Targets

Cat.	Target	Target Description	ENST ID	Trans. SEQ ID NO	ENSP ID	Prot. SEQ ID NO	OPT. SEQ ID NO
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide	238081	156	238081	1321	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide	248975	157	248975	1322	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	264335	158	264335	1323	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide	307630	159	306330	1324	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	353245	160	309503	1325	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide	353703	161	300161	1326	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide	372839	162	361930	1327	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide	381844	163	371267	1328	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	395948	164	379278	1329	
AS	14-3-3	tyrosine 3-	395951	165	379281	1330	

		monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide					
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	395953	166	379283	133 1	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	395956	167	379286	1332	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	395957	168	379287	1333	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	395958	169	379288	1334	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	4 1413 1	170	406058	1335	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	4 18997	171	4 1655 1	1336	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	419477	172	395 114	1337	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide	428262	173	394729	1338	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	437293	174	394880	1339	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide	445830	175	394558	1340	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide	446619	176	398990	1341	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide	453207	177	390645	1342	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	457309	178	398599	1343	

AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	517797	179	427801	1344	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	521309	180	429623	1345	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	521328	181	429041	1346	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	521607	182	430058	1347	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	522542	183	430072	1348	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	522819	184	428775	1349	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	523131	185	428381	1350	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	523848	186	428860	1351	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide	536755	187	443803	1352	
AS	14-3-3	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide	539979	188	443226	1353	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 1	287295	189	287295	1354	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 2	307864	190	312370	1355	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 1	319908	191	315122	1356	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 3	333607	192	327671	1357	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 3	335375	193	335369	1358	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 1	346424	194	316320	1359	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 2	373248	195	362345	1360	
AS	AIF	apoptosis-inducing factor,	395039	196	378480	1361	

		mitochondrion-associated, 2					
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 3	399163	197	382116	1362	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 3	399167	198	382120	1363	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 3	405089	199	385800	1364	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 3	434714	200	399657	1365	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 3	440238	201	390798	1366	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 1	440263	202	405879	1367	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 3	441376	203	402067	1368	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 1	460436	204	431222	1369	
AS	AIF	apoptosis-inducing factor, mitochondrion-associated, 1	535724	205	446113	1370	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	263826	206	263826	1371	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 2	311278	207	309428	1372	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	336199	208	336943	1373	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 1	349310	209	270202	1374	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 2	358335	210	351095	1375	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	366539	211	355497	1376	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	366540	212	355498	1377	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 2	391844	213	375719	1378	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 2	392037	214	375891	1379	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 2	392038	215	375892	1380	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 1	402615	216	385326	1381	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 1	407796	217	384293	1382	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 2	416362	218	407999	1383	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 2	416994	219	392458	1384	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 2	423127	220	403842	1385	
AS	AKT	v-akt murine thymoma viral	424901	221	399532	1386	

	(PKB)	oncogene homolog 2					
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 2	427375	222	403890	1387	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 2	452077	223	404083	1388	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 2	456441	224	396532	1389	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 2	537834	225	441591	1390	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 1	544168	226	443897	1391	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	552631	227	447820	1392	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 1	554581	228	451828	1393	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 1	554848	229	451166	1394	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 1	555528	230	450688	1395	
AS	AKT (PKB)	v-akt murine thymoma viral oncogene homolog 1	555926	231	451824	1396	
AS	ANT	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	281456	232	281456	1397	
AS	Apaf-1	apoptotic peptidase activating factor 1	333991	233	334558	1398	
AS	Apaf-1	apoptotic peptidase activating factor 1	339433	234	341830	1399	
AS	Apaf-1	apoptotic peptidase activating factor 1	357310	235	349862	1400	
AS	Apaf-1	apoptotic peptidase activating factor 1	359972	236	353059	1401	
AS	Apaf-1	apoptotic peptidase activating factor 1	547045	237	449791	1402	
AS	Apaf-1	apoptotic peptidase activating factor 1	549007	238	448161	1403	
AS	Apaf-1	apoptotic peptidase activating factor 1	550527	239	448449	1404	
AS	Apaf-1	apoptotic peptidase activating factor 1	551964	240	448165	1405	
AS	Apaf-1	apoptotic peptidase activating factor 1	552268	241	448826	1406	
AS	APRIL(TNFSF13)	tumor necrosis factor (ligand) superfamily, member 13	338784	242	343505	1407	
AS	APRIL(TNFSF13)	tumor necrosis factor (ligand) superfamily, member 13	349228	243	314455	1408	
AS	APRIL(TNFSF13)	tumor necrosis factor (ligand) superfamily, member 13	380535	244	369908	1409	
AS	APRIL(	tumor necrosis factor (ligand)	396545	245	379794	1410	

	TNFSF13)	superfamily, member 13					
AS	ARTS	phosphoribosyl pyrophosphate synthetase 1	372418	246	361495	1411	
AS	ARTS	phosphoribosyl pyrophosphate synthetase 1	372419	247	361496	1412	
AS	ARTS	phosphoribosyl pyrophosphate synthetase 1	372428	248	361505	1413	
AS	ARTS	phosphoribosyl pyrophosphate synthetase 1	372435	249	361512	1414	
AS	ARTS	phosphoribosyl pyrophosphate synthetase 1	543248	250	443185	1415	
AS	ASK1 (MAP3K5)	mitogen-activated protein kinase kinase kinase 5	355845	251	348104	1416	
AS	ASK1 (MAP3K5)	mitogen-activated protein kinase kinase kinase 5	359015	252	351908	1417	
AS	ASK1 (MAP3K5)	mitogen-activated protein kinase kinase kinase 5	367768	253	356742	1418	
AS	BAD	BCL2-associated agonist of cell death	309032	254	309103	1419	
AS	BAD	BCL2-associated agonist of cell death	394532	255	378040	1420	
AS	BAD	BCL2-associated agonist of cell death	540152	256	440807	1421	
AS	BAFF(TNFSF13B)	tumor necrosis factor (ligand) superfamily, member 13b	375887	257	365048	1422	
AS	BAFF(TNFSF13B)	tumor necrosis factor (ligand) superfamily, member 13b	430559	258	389540	1423	
AS	BAFF(TNFSF13B)	tumor necrosis factor (ligand) superfamily, member 13b	542136	259	445334	1424	
AS	Bak	BCL2-antagonist/killer 1	360661	260	353878	1425	
AS	Bak	BCL2-antagonist/killer 1	374460	261	363584	1426	
AS	Bak	BCL2-antagonist/killer 1	374467	262	363591	1427	
AS	Bak	BCL2-antagonist/killer 1	442998	263	391258	1428	
AS	BAX	BCL2-associated X protein	293288	264	293288	1429	
AS	BAX	BCL2-associated X protein	345358	265	263262	1430	
AS	BAX	BCL2-associated X protein	354470	266	346461	1431	
AS	BAX	BCL2-associated X protein	391871	267	375744	1432	
AS	BAX	BCL2-associated X protein	415969	268	389971	1433	
AS	BAX	BCL2-associated X protein	539787	269	441413	1434	
AS	Bcl-2	B-cell CLL/lymphoma 2	333681	270	329623	1435	
AS	Bcl-2	B-cell CLL/lymphoma 2	398117	271	381185	1436	
AS	Bcl-2	B-cell CLL/lymphoma 2	444484	272	404214	1437	
AS	Bcl-B	BCL2-like 10 (apoptosis facilitator)	260442	273	260442	1438	

AS	Bcl-W	BCL2-like 2	250405	274	250405	1439	
AS	Bcl-W	BCL2-like 2	554635	275	451234	1440	
AS	Bcl-W	BCL2-like 2	557236	276	451701	1441	
AS	Bcl-W	BCL2-like 2	557579	277	452265	1442	
AS	Bcl-XL	BCL2-like 1	307677	278	302564	1443	
AS	Bcl-XL	BCL2-like 1	376055	279	365223	1444	
AS	Bcl-XL	BCL2-like 1	376062	280	365230	1445	
AS	Bcl-XL	BCL2-like 1	420488	281	390760	1446	
AS	Bcl-XL	BCL2-like 1	420653	282	405563	1447	
AS	Bcl-XL	BCL2-like 1	422920	283	411252	1448	
AS	Bcl-XL	BCL2-like 1	439267	284	389688	1449	
AS	Bcl-XL	BCL2-like 1	450273	285	406203	1450	
AS	Bcl-XL	BCL2-like 1	456404	286	395545	1451	
AS	BCMA	tumor necrosis factor receptor superfamily, member 17	53243	287	53243	1452	
AS	BCMA	tumor necrosis factor receptor superfamily, member 17	396495	288	379753	1453	
AS	BCMA	tumor necrosis factor receptor superfamily, member 17	435355	289	401782	1454	
AS	BFL1	BCL2-related protein A1	267953	290	267953	1455	
AS	BFL1	BCL2-related protein A1	335661	291	335250	1456	
AS	Bid	BH3 interacting domain death agonist	317361	292	318822	1457	
AS	Bid	BH3 interacting domain death agonist	342111	293	344594	1458	
AS	Bid	BH3 interacting domain death agonist	399765	294	382667	1459	
AS	Bid	BH3 interacting domain death agonist	399767	295	382669	1460	
AS	Bid	BH3 interacting domain death agonist	399774	296	382674	1461	
AS	Bid	BH3 interacting domain death agonist	551952	297	449236	1462	
AS	Bik	BCL2-interacting killer (apoptosis-inducing)	216115	298	216115	1463	
AS	Bim	BCL2-like 11 (apoptosis facilitator)	308659	299	309226	1464	
AS	Bim	BCL2-like 11 (apoptosis facilitator)	337565	300	338374	1465	
AS	Bim	BCL2-like 11 (apoptosis facilitator)	357757	301	350398	1466	
AS	Bim	BCL2-like 11 (apoptosis facilitator)	393252	302	376941	1467	
AS	Bim	BCL2-like 11 (apoptosis facilitator)	393253	303	376942	1468	
AS	Bim	BCL2-like 11 (apoptosis facilitator)	393256	304	376943	1469	
AS	Bim	BCL2-like 11 (apoptosis facilitator)	432179	305	411870	1470	
AS	Bim	BCL2-like 11 (apoptosis	452033	306	403666	1471	

		facilitator)					
AS	BMF	Bcl2 modifying factor	220446	307	220446	1472	
AS	BMF	Bcl2 modifying factor	354670	308	346697	1473	
AS	BMF	Bcl2 modifying factor	397573	309	380703	1474	
AS	BMF	Bcl2 modifying factor	431415	310	396511	1475	
AS	BMF	Bcl2 modifying factor	559701	311	453919	1476	
AS	BMF	Bcl2 modifying factor	561282	312	453522	1477	
AS	BMF	Bcl2 modifying factor	561360	313	453892	1478	
AS	BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)	342045	314	339371	1479	
AS	BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)	344773	315	343412	1480	
AS	BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)	361704	316	354699	1481	
AS	BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)	379623	317	368944	1482	
AS	BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)	379624	318	368945	1483	
AS	BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)	379632	319	368953	1484	
AS	BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)	436924	320	392345	1485	
AS	Calcineurin A	protein phosphatase 3, catalytic subunit, alpha isozyme	323055	321	320580	1486	
AS	Calcineurin A	protein phosphatase 3, catalytic subunit, alpha isozyme	394853	322	378322	1487	
AS	Calcineurin A	protein phosphatase 3, catalytic subunit, alpha isozyme	394854	323	378323	1488	
AS	Calcineurin A	protein phosphatase 3, catalytic subunit, alpha isozyme	507176	324	422990	1489	
AS	Calcineurin A	protein phosphatase 3, catalytic subunit, alpha isozyme	512215	325	422781	1490	
AS	Calcineurin A	protein phosphatase 3, catalytic subunit, alpha isozyme	523694	326	429350	1491	
AS	Calcineurin A	protein phosphatase 3, catalytic subunit, alpha isozyme	525819	327	434599	1492	
AS	Calcineurin A	protein phosphatase 3, catalytic subunit, alpha isozyme	529324	328	431619	1493	
AS	Caspase-1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	353247	329	344132	1494	
AS	Caspase-1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	393136	330	376844	1495	
AS	Caspase-	caspase 1, apoptosis-related	415981	331	408446	1496	

	1	cysteine peptidase (interleukin 1, beta, convertase)					
AS	Caspase-1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	436863	332	410076	1497	
AS	Caspase-1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	446369	333	403260	1498	
AS	Caspase-1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	525825	334	434779	1499	
AS	Caspase-1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	526568	335	434250	1500	
AS	Caspase-1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	528974	336	434259	1501	
AS	Caspase-1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	529871	337	431947	1502	
AS	Caspase-1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	531166	338	434303	1503	
AS	Caspase-1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	533400	339	433138	1504	
AS	Caspase-1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	534497	340	436875	1505	
AS	Caspase-10	caspase 10, apoptosis-related cysteine peptidase	272879	341	272879	1506	
AS	Caspase-10	caspase 10, apoptosis-related cysteine peptidase	286186	342	286186	1507	
AS	Caspase-10	caspase 10, apoptosis-related cysteine peptidase	346817	343	237865	1508	
AS	Caspase-10	caspase 10, apoptosis-related cysteine peptidase	360132	344	353250	1509	
AS	Caspase-2	caspase 2, apoptosis-related cysteine peptidase	310447	345	312664	1510	
AS	Caspase-2	caspase 2, apoptosis-related cysteine peptidase	350623	346	340030	1511	
AS	Caspase-2	caspase 2, apoptosis-related cysteine peptidase	392923	347	376654	1512	
AS	Caspase-3	caspase 3, apoptosis-related cysteine peptidase	308394	348	311032	1513	
AS	Caspase-3	caspase 3, apoptosis-related cysteine peptidase	438467	349	390792	1514	
AS	Caspase-3	caspase 3, apoptosis-related cysteine peptidase	447121	350	407142	1515	
AS	Caspase-3	caspase 3, apoptosis-related cysteine peptidase	523916	351	428929	1516	
AS	Caspase-4	caspase 4, apoptosis-related cysteine peptidase	355546	352	347741	1517	
AS	Caspase-4	caspase 4, apoptosis-related cysteine peptidase	417440	353	401673	1518	

AS	Caspase-4	caspase 4, apoptosis-related cysteine peptidase	444739	354	388566	15 19	
AS	Caspase-5	caspase 5, apoptosis-related cysteine peptidase	2603 15	355	2603 15	1520	
AS	Caspase-5	caspase 5, apoptosis-related cysteine peptidase	393 139	356	376847	1521	
AS	Caspase-5	caspase 5, apoptosis-related cysteine peptidase	393 141	357	376849	1522	
AS	Caspase-5	caspase 5, apoptosis-related cysteine peptidase	4 18434	358	398130	1523	
AS	Caspase-5	caspase 5, apoptosis-related cysteine peptidase	444749	359	388365	1524	
AS	Caspase-5	caspase 5, apoptosis-related cysteine peptidase	526056	360	436877	1525	
AS	Caspase-5	caspase 5, apoptosis-related cysteine peptidase	53 1367	361	434471	1526	
AS	Caspase-6	caspase 6, apoptosis-related cysteine peptidase	265 164	362	265 164	1527	
AS	Caspase-6	caspase 6, apoptosis-related cysteine peptidase	352981	363	285333	1528	
AS	Caspase-7	caspase 7, apoptosis-related cysteine peptidase	345633	364	298701	1529	
AS	Caspase-7	caspase 7, apoptosis-related cysteine peptidase	3693 15	365	358321	1530	
AS	Caspase-7	caspase 7, apoptosis-related cysteine peptidase	3693 16	366	358322	153 1	
AS	Caspase-7	caspase 7, apoptosis-related cysteine peptidase	3693 18	367	358324	1532	
AS	Caspase-7	caspase 7, apoptosis-related cysteine peptidase	3693 19	368	358325	1533	
AS	Caspase-7	caspase 7, apoptosis-related cysteine peptidase	369321	369	358327	1534	
AS	Caspase-7	caspase 7, apoptosis-related cysteine peptidase	36933 1	370	358337	1535	
AS	Caspase-7	caspase 7, apoptosis-related cysteine peptidase	429617	371	400094	1536	
AS	Caspase-7	caspase 7, apoptosis-related cysteine peptidase	442393	372	394482	1537	
AS	Caspase-7	caspase 7, apoptosis-related cysteine peptidase	452490	373	398107	1538	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	264274	374	264274	1539	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	264275	375	264275	1540	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	323492	376	325722	1541	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	358485	377	35 1273	1542	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	392258	378	376087	1543	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	392259	379	376088	1544	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	392261	380	376089	1545	

AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	392263	381	376091	1546	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	392266	382	376094	1547	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	413726	383	397528	1548	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	429881	384	390641	1549	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	432109	385	412523	1550	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	440732	386	396869	1551	
AS	Caspase-8	caspase 8, apoptosis-related cysteine peptidase	447616	387	388306	1552	
AS	Caspase-9	caspase 9, apoptosis-related cysteine peptidase	333868	388	330237	1553	
AS	Caspase-9	caspase 9, apoptosis-related cysteine peptidase	348549	389	255256	1554	
AS	Caspase-9	caspase 9, apoptosis-related cysteine peptidase	375874	390	365034	1555	
AS	Caspase-9	caspase 9, apoptosis-related cysteine peptidase	375890	391	365051	1556	
AS	Caspase-9	caspase 9, apoptosis-related cysteine peptidase	440484	392	411304	1557	
AS	Caspase-9	caspase 9, apoptosis-related cysteine peptidase	447522	393	396540	1558	
AS	Caspase-9	caspase 9, apoptosis-related cysteine peptidase	546424	394	449584	1559	
AS	CD27	CD27 molecule	266557	395	266557	1560	
AS	CD30	tumor necrosis factor receptor superfamily, member 8	263932	396	263932	1561	
AS	CD30	tumor necrosis factor receptor superfamily, member 8	413146	397	398337	1562	
AS	CD30	tumor necrosis factor receptor superfamily, member 8	417814	398	390650	1563	
AS	CD30L	tumor necrosis factor (ligand) superfamily, member 8	223795	399	223795	1564	
AS	CD40	CD40 molecule, TNF receptor superfamily member 5	372278	400	361352	1565	
AS	CD40L(TNFSF5)	CD40 ligand	370628	401	359662	1566	
AS	CD40L(TNFSF5)	CD40 ligand	370629	402	359663	1567	
AS	CD41	CD40 molecule, TNF receptor superfamily member 5	372276	403	361350	1568	
AS	CD42	CD40 molecule, TNF receptor superfamily member 5	372285	404	361359	1569	
AS	CD70(TNFSF7)	CD70 molecule	245903	405	245903	1570	
AS	CD70(TNFSF7)	CD70 molecule	423145	406	395294	1571	
AS	CDK1	cyclin-dependent kinase 1	316629	407	325970	1572	

	(p34)						
AS	CDK1 (p34)	cyclin-dependent kinase 1	373809	408	362915	1573	
AS	CDK1 (p34)	cyclin-dependent kinase 1	395284	409	378699	1574	
AS	CDK1 (p34)	cyclin-dependent kinase 1	448257	410	397973	1575	
AS	CDK1 (p34)	cyclin-dependent kinase 1	519078	411	430665	1576	
AS	CDK5	cyclin-dependent kinase 5	485972	412	419782	1577	
AS	CDK5R 1 (p35)	cyclin-dependent kinase 5, regulatory subunit 1 (p35)	313401	413	318486	1578	
AS	c-FLIP(S)	CASP8 and FADD-like apoptosis regulator	309955	414	312455	1579	
AS	c-FLIP(S)	CASP8 and FADD-like apoptosis regulator	340870	415	339326	1580	
AS	c-FLIP(S)	CASP8 and FADD-like apoptosis regulator	343375	416	339391	1581	
AS	c-FLIP(S)	CASP8 and FADD-like apoptosis regulator	355558	417	347757	1582	
AS	c-FLIP(S)	CASP8 and FADD-like apoptosis regulator	395148	418	378580	1583	
AS	c-FLIP(S)	CASP8 and FADD-like apoptosis regulator	417748	419	412882	1584	
AS	c-FLIP(S)	CASP8 and FADD-like apoptosis regulator	423241	420	399420	1585	
AS	c-FLIP(S)	CASP8 and FADD-like apoptosis regulator	433445	421	391029	1586	
AS	c-FLIP(S)	CASP8 and FADD-like apoptosis regulator	441224	422	411897	1587	
AS	c-FLIP(S)	CASP8 and FADD-like apoptosis regulator	443227	423	413270	1588	
AS	cIAP1	baculoviral IAP repeat containing 3	NA	424	NA	1589	2488
AS	c-IAP1	baculoviral IAP repeat containing 3	263464	425	263464	1590	
AS	c-IAP1	baculoviral IAP repeat containing 3	532808	426	432907	1591	
AS	cIAP2	baculoviral IAP repeat containing 2	NA	427	NA	1592	
AS	C-IAP2	baculoviral IAP repeat containing 2	227758	428	227758	1593	
AS	C-IAP2	baculoviral IAP repeat containing 2	530675	429	431723	1594	
AS	C-IAP2	baculoviral IAP repeat containing 2	532672	430	434979	1595	
AS	C-IAP2	baculoviral IAP repeat containing 2	541741	431	440771	1596	
AS	c-Jun	jun proto-oncogene	371222	432	360266	1597	
AS	c-Raf-1	v-raf-1 murine leukemia viral oncogene homolog 1	251849	433	251849	1598	
AS	c-Raf-1	v-raf-1 murine leukemia viral oncogene homolog 1	442415	434	401888	1599	

AS	c-Raf-1	v-raf- 1 murine leukemia viral oncogene homolog 1	534997	435	441 186	1600	
AS	c-Raf-1	v-raf- 1 murine leukemia viral oncogene homolog 1	542177	436	443567	1601	
AS	Cytochr ome c	cytochrome c, somatic	305786	437	307786	1602	
AS	Cytochr ome c	cytochrome c, somatic	409409	438	386270	1603	
AS	Cytochr ome c	cytochrome c, somatic	409764	439	387279	1604	
AS	Cytochr ome c	cytochrome c, somatic	413447	440	416479	1605	
AS	DAXX	death-domain associated protein	266000	441	266000	1606	
AS	DAXX	death-domain associated protein	374542	442	363668	1607	
AS	DAXX	death-domain associated protein	383062	443	372539	1608	
AS	DAXX	death-domain associated protein	383 194	444	372681	1609	
AS	DAXX	death-domain associated protein	399060	445	382014	1610	
AS	DAXX	death-domain associated protein	399344	446	382281	161 1	
AS	DAXX	death-domain associated protein	414083	447	396876	1612	
AS	DAXX	death-domain associated protein	414272	448	409756	1613	
AS	DAXX	death-domain associated protein	419855	449	397612	1614	
AS	DAXX	death-domain associated protein	428268	450	408215	1615	
AS	DAXX	death-domain associated protein	42953 1	45 1	415898	1616	
AS	DAXX	death-domain associated protein	433482	452	404623	1617	
AS	DAXX	death-domain associated protein	4363 11	453	404376	1618	
AS	DAXX	death-domain associated protein	438332	454	4 11700	1619	
AS	DAXX	death-domain associated protein	440500	455	403986	1620	
AS	DAXX	death-domain associated protein	445009	456	394108	1621	
AS	DAXX	death-domain associated protein	446403	457	406008	1622	
AS	DAXX	death-domain associated protein	453407	458	408499	1623	
AS	DAXX	death-domain associated protein	45393 1	459	412433	1624	
AS	DAXX	death-domain associated protein	454197	460	412177	1625	
AS	DAXX	death-domain associated protein	455860	461	410772	1626	

AS	DAXX	death-domain associated protein	547663	462	4471 15	1627	
AS	DAXX	death-domain associated protein	548604	463	448337	1628	
AS	DAXX	death-domain associated protein	550822	464	447861	1629	
AS	DAXX	death-domain associated protein	552944	465	447833	1630	
AS	DcR3	tumor necrosis factor receptor superfamily, member 6b, decoy	342852	466	342328	163 1	
AS	DcR3	tumor necrosis factor receptor superfamily, member 6b, decoy	369996	467	359013	1632	
AS	DcR3	tumor necrosis factor receptor superfamily, member 6b, decoy	370006	468	359023	1633	
AS	DFF40 (CAD)	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase)	338895	469	339524	1634	
AS	DFF40 (CAD)	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase)	339350	470	34321 8	1635	
AS	DFF40 (CAD)	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase)	341385	471	345906	1636	
AS	DFF40 (CAD)	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase)	378206	472	367448	1637	
AS	DFF40 (CAD)	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase)	378209	473	367454	1638	
AS	DFF40 (CAD)	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase)	378212	474	367457	1639	
AS	DFF40 (CAD)	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase)	430539	475	389502	1640	
AS	DFF40 (CAD)	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase)	448632	476	4 11635	1641	
AS	DFF40 (CAD)	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase)	491998	477	436775	1642	
AS	DR3	tumor necrosis factor receptor superfamily, member 25	348333	478	3 1445 1	1643	
AS	DR3	tumor necrosis factor receptor superfamily, member 25	35 1748	479	326762	1644	
AS	DR3	tumor necrosis factor receptor superfamily, member 25	35 1959	480	337713	1645	
AS	DR3	tumor necrosis factor receptor superfamily, member 25	356876	481	349341	1646	
AS	DR3	tumor necrosis factor receptor superfamily, member 25	377782	482	367013	1647	
AS	DR4	tumor necrosis factor receptor	221 132	483	221 132	1648	

		superfamily, member 10a					
AS	DR5	tumor necrosis factor receptor superfamily, member 10b	276431	484	276431	1649	
AS	DR5	tumor necrosis factor receptor superfamily, member 10b	347739	485	317859	1650	
AS	DR5	tumor necrosis factor receptor superfamily, member 10b	542226	486	443386	1651	
AS	DR6	tumor necrosis factor receptor superfamily, member 21	296861	487	296861	1652	
AS	DR6	tumor necrosis factor receptor superfamily, member 21	419206	488	390032	1653	
AS	EGFR	epidermal growth factor receptor	275493	489	275493	1654	
AS	EGFR	epidermal growth factor receptor	342916	490	342376	1655	
AS	EGFR	epidermal growth factor receptor	344576	491	345973	1656	
AS	EGFR	epidermal growth factor receptor	395504	492	378880	1657	
AS	EGFR	epidermal growth factor receptor	420316	493	413843	1658	
AS	EGFR	epidermal growth factor receptor	442591	494	410031	1659	
AS	EGFR	epidermal growth factor receptor	454757	495	395243	1660	
AS	EGFR	epidermal growth factor receptor	455089	496	415559	1661	
AS	EGFR	epidermal growth factor receptor	533450	497	435262	1662	
AS	ErbB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	269571	498	269571	1663	
AS	ErbB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	406381	499	385185	1664	
AS	ErbB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	445658	500	404047	1665	
AS	ErbB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	540042	501	446382	1666	
AS	ErbB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	540147	502	443562	1667	

AS	ErbB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	541774	503	446466	1668	
AS	ErbB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	267101	504	267101	1669	
AS	ErbB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	394099	505	377659	1670	
AS	ErbB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	411731	506	415753	1671	
AS	ErbB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	415288	507	408340	1672	
AS	ErbB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	450146	508	399178	1673	
AS	ErbB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	549282	509	448636	1674	
AS	ErbB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	551085	510	448483	1675	
AS	Erk(MAPKl/3)	mitogen-activated protein kinase 1	215832	511	215832	1676	
AS	Erk(MAPKl/3)	mitogen-activated protein kinase 3	263025	512	263025	1677	
AS	Erk(MAPKl/3)	mitogen-activated protein kinase 3	322266	513	327293	1678	
AS	Erk(MAPKl/3)	mitogen-activated protein kinase 3	395200	514	378626	1679	
AS	Erk(MAPKl/3)	mitogen-activated protein kinase 3	395202	515	378628	1680	
AS	Erk(MAPKl/3)	mitogen-activated protein kinase 1	398822	516	381803	1681	
AS	Erk(MAPKl/3)	mitogen-activated protein kinase 3	403394	517	384895	1682	
AS	Erk(MAPKl/3)	mitogen-activated protein kinase 1	415911	518	409149	1683	
AS	Erk(MAPKl/3)	mitogen-activated protein kinase 3	484663	519	432742	1684	
AS	Erk(MAPKl/3)	mitogen-activated protein kinase 1	544786	520	440842	1685	
AS	FADD	Fas (TNFRSF6)-associated via death domain	301838	521	301838	1686	
AS	FLASH	caspase 8 associated protein 2	237177	522	NA	1687	
AS	FLASH	caspase 8 associated protein 2	419040	523	NA		
AS	FLASH	caspase 8 associated protein 2	444163	524	NA		
AS	FLASH	caspase 8 associated protein 2	547893	525	NA		
AS	FLASH	caspase 8 associated protein 2	548224	526	NA		

AS	FLASH	caspase 8 associated protein 2	551025	527	NA		
AS	FLASH	caspase 8 associated protein 2	552401	528	NA		
AS	FN14	tumor necrosis factor receptor superfamily, member 12A	326577	529	326737	1688	
AS	FN14	tumor necrosis factor receptor superfamily, member 12A	341627	530	343894	1689	
AS	GCK (MAP4 K2)	mitogen-activated protein kinase kinase kinase kinase 2	294066	531	294066	1690	
AS	GRB2	growth factor receptor-bound protein 2	316615	532	317360	1691	
AS	GRB2	growth factor receptor-bound protein 2	316804	533	339007	1692	
AS	GRB2	growth factor receptor-bound protein 2	392562	534	376345	1693	
AS	GRB2	growth factor receptor-bound protein 2	392564	535	376347	1694	
AS	H-Ras	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	311189	536	309845	1695	
AS	H-Ras	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	397594	537	380722	1696	
AS	H-Ras	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	397596	538	380723	1697	
AS	H-Ras	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	417302	539	388246	1698	
AS	H-Ras	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	451590	540	407586	1699	
AS	H-Ras	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	493230	541	434023	1700	
AS	HRK	harakiri, BCL2 interacting protein (contains only BH3 domain)	257572	542	257572	1701	
AS	HSP27	heat shock 27kDa protein 1	248553	543	248553	1702	
AS	HSP27	heat shock 27kDa protein 3	302005	544	303394	1703	
AS	HSP27	Heat shock protein beta-2	304298	545	302476	1704	
AS	HSP27	heat shock 27kDa protein 1	432276	546	406545	1705	
AS	HSP27	Heat shock protein beta-2	537382	547	445585	1706	
AS	HtrA2/Omi	HtrA serine peptidase 2	258080	548	258080	1707	
AS	HtrA2/Omi	HtrA serine peptidase 2	352222	549	312893	1708	
AS	Humanin	MT-RNR2-like 4	399974	550	382856	1709	
AS	Humanin	MT-RNR2-like 5	512524	551	437910	1710	
AS	Humanin	MT-RNR2-like 8	536684	552	439666	1711	
AS	Humanin	MT-RNR2-like 1	540040	553	439228	1712	
AS	Humanin	MT-RNR2-like 3	543500	554	443339	1713	
AS	Humani	MT-RNR2-like 7	544824	555	439985	1714	

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AS	Humani n	MT-RNR2-like 10	545075	556	442159	1715	
AS	Humani n	MT-RNR2-like 6	570419	557	461075	1716	
AS	ICAD	DNA fragmentation factor, 45kDa, alpha polypeptide	377036	558	366235	1717	
AS	ICAD	DNA fragmentation factor, 45kDa, alpha polypeptide	377038	559	366237	1718	
AS	IGF-1R	insulin-like growth factor 1 receptor	268035	560	268035	1719	
AS	IKK (alpha)	conserved helix-loop-helix ubiquitous kinase	370397	561	359424	1720	
AS	IKK (beta)	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	379708	562	369030	1721	
AS	IKK (beta)	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	416505	563	404920	1722	
AS	IKK (beta)	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	520810	564	430684	1723	
AS	IKK-gamma	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	263518	565	263518	1724	
AS	IKK-gamma	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	369601	566	358614	1725	
AS	IKK-gamma	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	369606	567	358619	1726	
AS	IKK-gamma	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	369607	568	358620	1727	
AS	IKK-gamma	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	369609	569	358622	1728	
AS	IKK-gamma	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	422680	570	390368	1729	
AS	IKK-gamma	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	440286	571	394934	1730	
AS	IKK-gamma	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	445622	572	395205	1731	
AS	IKK-gamma	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	455588	573	400769	1732	
AS	IRAKI	interleukin- 1 receptor-associated kinase 1	369980	574	358997	1733	
AS	IRAKI	interleukin- 1 receptor-associated kinase 1	393682	575	377287	1734	
AS	IRAKI	interleukin- 1 receptor-associated kinase 1	393687	576	377291	1735	

AS	IRAKI	interleukin- 1 receptor-associated kinase 1	429936	577	392662	1736	
AS	IRAK2	interleukin- 1 receptor-associated kinase 2	256458	578	256458	1737	
AS	IRS-1	insulin receptor substrate 1	305 123	579	304895	1738	
AS	jBid; formed after cleaving BID at position 25	jBID	NA		NA	1739	
AS	JNK 1(MAPK8)	mitogen-activated protein kinase 8	360332	580	353483	1740	
AS	JNK 1(MAPK8)	mitogen-activated protein kinase 8	374174	581	363289	1741	
AS	JNK 1(MAPK8)	mitogen-activated protein kinase 8	374176	582	363291	1742	
AS	JNK 1(MAPK8)	mitogen-activated protein kinase 8	374179	583	363294	1743	
AS	JNK 1(MAPK8)	mitogen-activated protein kinase 8	3741 82	584	363297	1744	
AS	JNK 1(MAPK8)	mitogen-activated protein kinase 8	3741 89	585	363304	1745	
AS	JNK 1(MAPK8)	mitogen-activated protein kinase 8	39561 1	586	378974	1746	
AS	JNK 1(MAPK8)	mitogen-activated protein kinase 8	426557	587	397729	1747	
AS	JNK 1(MAPK8)	mitogen-activated protein kinase 8	429041	588	393223	1748	
AS	JNK 1(MAPK8)	mitogen-activated protein kinase 8	432379	589	387936	1749	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	359221	590	352157	1750	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	361569	591	355297	1751	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	395 157	592	378586	1752	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	395 160	593	378589	1753	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	395 161	594	378590	1754	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	395 166	595	378595	1755	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	395 169	596	378598	1756	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	449047	597	414469	1757	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	502302	598	42391 8	1758	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	50391 1	599	421409	1759	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	506773	600	421359	1760	

AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	509464	601	424128	1761	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	511167	602	422277	1762	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	511328	603	421762	1763	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	512017	604	424755	1764	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	512564	605	422985	1765	
AS	JNK3(MAPK10)	mitogen-activated protein kinase 10	515400	606	424154	1766	
AS	MAP1	mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	169293	607	169293	1767	
AS	MAP1	mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	296280	608	296280	1768	
AS	MAP1	mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	337774	609	336792	1769	
AS	MAP1	mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	392472	610	376264	1770	
AS	MAP1	mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	541811	611	440446	1771	
AS	MAP1	mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	541896	612	446240	1772	
AS	Mcl-1	myeloid cell leukemia sequence 1 (BCL2-related)	307940	613	309973	1773	
AS	Mcl-1	myeloid cell leukemia sequence 1 (BCL2-related)	369026	614	358022	1774	
AS	Mcl-1	myeloid cell leukemia sequence 1 (BCL2-related)	439749	615	411395	1775	
AS	MEK1(MAP2K1)	mitogen-activated protein kinase 1	215832	616	215832	1776	
AS	MEK1(MAP2K1)	mitogen-activated protein kinase kinase 1	307102	617	302486	1777	
AS	MEK1(MAP2K1)	mitogen-activated protein kinase 1	415911	618	409149	1778	
AS	MEK1(MAP2K1)	mitogen-activated protein kinase 1	544786	619	440842	1779	
AS	MEK2(	mitogen-activated protein	262948	620	262948	1780	

	MAP2K 2)	kinase kinase 2					
AS	MEK4 (MAP2 K4)	mitogen-activated protein kinase kinase 4	353533	621	262445	1781	
AS	MEK4 (MAP2 K4)	mitogen-activated protein kinase kinase 4	415385	622	410402	1782	
AS	MEK4 (MAP2 K4)	mitogen-activated protein kinase kinase 4	536413	623	441610	1783	
AS	MEK4 (MAP2 K4)	mitogen-activated protein kinase kinase 4	538465	624	444874	1784	
AS	MEKK1 (MAP3 K1)	mitogen-activated protein kinase kinase kinase 1	399503	625	382423	1785	
AS	NADE (NGFR AP1)	nerve growth factor receptor (TNFRSF16) associated protein 1	299872	626	299872	1786	
AS	NADE (NGFR AP1)	nerve growth factor receptor (TNFRSF16) associated protein 1	361298	627	354843	1787	
AS	NADE (NGFR AP1)	nerve growth factor receptor (TNFRSF16) associated protein 1	372634	628	361717	1788	
AS	NADE (NGFR AP1)	nerve growth factor receptor (TNFRSF16) associated protein 1	372635	629	361718	1789	
AS	NADE (NGFR AP1)	nerve growth factor receptor (TNFRSF16) associated protein 1	372645	630	361728	1790	
AS	NGF	nerve growth factor (beta polypeptide)	369512	631	358525	1791	
AS	NGFR	nerve growth factor receptor	172229	632	172229	1792	
AS	NGFR	nerve growth factor receptor	504201	633	421731	1793	
AS	NIK (MAP3 K14)	mitogen-activated protein kinase kinase kinase 14	344686	634	342059	1794	
AS	NIK (MAP3 K14)	mitogen-activated protein kinase kinase kinase 14	376926	635	366125	1795	
AS	NOXA	phorbol-12-myristate-13-acetate-induced protein 1	269518	636	269518	1796	
AS	NOXA	phorbol-12-myristate-13-acetate-induced protein 1	316660	637	326119	1797	
AS	OX40	tumor necrosis factor receptor superfamily, member 4	379236	638	368538	1798	
AS	OX40	tumor necrosis factor receptor superfamily, member 4	453580	639	390907	1799	
AS	OX40L(TNFSF4)	tumor necrosis factor (ligand) superfamily, member 4	281834	640	281834	1800	
AS	OX40L(	tumor necrosis factor (ligand)	367718	641	356691	1801	

	TNFSF4)	superfamily, member 4					
AS	OX40L(TNFSF4)	tumor necrosis factor (ligand) superfamily, member 4	545292	642	439704	1802	
AS	p53	tumor protein p53	269305	643	269305	1803	
AS	p53	tumor protein p53	269305	644	269305	1804	2489
AS	p53	tumor protein p53	359597	645	352610	1805	
AS	p53	tumor protein p53	396473	646	379735	1806	
AS	p53	tumor protein p53	413465	647	410739	1807	
AS	p53	tumor protein p53	414315	648	394195	1808	
AS	p53	tumor protein p53	419024	649	402130	1809	
AS	p53	tumor protein p53	420246	650	391127	1810	
AS	p53	tumor protein p53	445888	651	391478	1811	2490
AS	p53	tumor protein p53	455263	652	398846	1812	
AS	p53	tumor protein p53	503591	653	426252	1813	
AS	p53	tumor protein p53	508793	654	424104	1814	
AS	p53	tumor protein p53	509690	655	425104	1815	
AS	p53	tumor protein p53	514944	656	423862	1816	
AS	p53	tumor protein p53	545858	657	437792	1817	
AS	p70 S6 kinase 1	ribosomal protein S6 kinase, 70kDa, polypeptide 1	225577	658	225577	1818	
AS	p70 S6 kinase 1	ribosomal protein S6 kinase, 70kDa, polypeptide 1	393021	659	376744	1819	
AS	p70 S6 kinase 1	ribosomal protein S6 kinase, 70kDa, polypeptide 1	406116	660	384335	1820	
AS	p70 S6 kinase 1	ribosomal protein S6 kinase, 70kDa, polypeptide 1	443572	661	441993	1821	
AS	p70 S6 kinase 2	ribosomal protein S6 kinase, 70kDa, polypeptide 2	312629	662	308413	1822	
AS	p70 S6 kinase 2	ribosomal protein S6 kinase, 70kDa, polypeptide 2	528964	663	432847	1823	
AS	p70 S6 kinase 2	ribosomal protein S6 kinase, 70kDa, polypeptide 2	539188	664	442949	1824	
AS	p90Rsk	ribosomal protein S6 kinase, 90kDa, polypeptide 1	374162	665	363277	1825	
AS	p90Rsk	ribosomal protein S6 kinase, 90kDa, polypeptide 1	374164	666	363279	1826	
AS	p90Rsk	ribosomal protein S6 kinase, 90kDa, polypeptide 1	374168	667	363283	1827	
AS	p90Rsk	ribosomal protein S6 kinase, 90kDa, polypeptide 1	403732	668	383967	1828	
AS	p90Rsk	ribosomal protein S6 kinase, 90kDa, polypeptide 1	530003	669	432281	1829	
AS	p90Rsk	ribosomal protein S6 kinase, 90kDa, polypeptide 1	531382	670	435412	1830	
AS	PAK2	p21 protein (Cdc42/Rac)-activated kinase 2	327134	671	314067	1831	
AS	PARP-1	poly (ADP-ribose) polymerase 1	366790	672	355755	1832	

AS	PARP-1	poly (ADP-ribose) polymerase 1	366791	673	355756	1833	
AS	PARP-1	poly (ADP-ribose) polymerase 1	366792	674	355757	1834	
AS	PARP-1	poly (ADP-ribose) polymerase 1	366794	675	355759	1835	
AS	PARP-1	poly (ADP-ribose) polymerase 1	432338	676	412774	1836	
AS	PDPK1	3-phosphoinositide dependent protein kinase- 1	342085	677	344220	1837	
AS	PDPK1	3-phosphoinositide dependent protein kinase- 1	354836	678	346895	1838	
AS	PDPK1	3-phosphoinositide dependent protein kinase- 1	441549	679	395357	1839	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, alpha polypeptide	263967	680	263967	1840	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, beta polypeptide	289153	681	289153	1841	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, gamma polypeptide	359195	682	352121	1842	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, delta polypeptide	360563	683	353766	1843	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, delta polypeptide	361110	684	354410	1844	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, delta polypeptide	377346	685	366563	1845	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, gamma polypeptide	440650	686	392258	1846	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, beta polypeptide	461451	687	420399	1847	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, alpha polypeptide	468036	688	417479	1848	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, beta polypeptide	477593	689	418143	1849	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, beta polypeptide	483968	690	419857	1850	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, beta polypeptide	493568	691	417869	1851	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, gamma polypeptide	496166	692	419260	1852	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, delta polypeptide	536656	693	446444	1853	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, delta polypeptide	543390	694	443811	1854	
AS	PI3K	phosphoinositide-3 -kinase, catalytic, beta polypeptide	544716	695	438259	1855	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, alpha	308677	696	309591	1856	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, alpha	350356	697	340940	1857	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	370679	698	359713	1858	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	370680	699	359714	1859	

AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	370681	700	359715	1860	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	370682	701	359716	1861	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	370684	702	359718	1862	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	370685	703	359719	1863	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	370688	704	359722	1864	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	370689	705	359723	1865	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, gamma	377276	706	366488	1866	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	394838	707	378314	1867	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	394839	708	378315	1868	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	413538	709	397175	1869	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	417530	710	399326	1870	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	432111	711	392275	1871	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	436133	712	390906	1872	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	446538	713	401252	1873	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, beta	450730	714	393654	1874	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, alpha	535695	715	441654	1875	
AS	PKA-cat	protein kinase, cAMP-dependent, catalytic, alpha	536649	716	440418	1876	
AS	PKC-delta	protein kinase C, delta	330452	717	331602	1877	
AS	PKC-delta	protein kinase C, delta	394729	718	378217	1878	
AS	PKC-delta	protein kinase C, delta	478843	719	419726	1879	
AS	PKC-delta	protein kinase C, delta	487897	720	418106	1880	
AS	PKC-Zeta	protein kinase C, zeta	378567	721	367830	1881	
AS	PKC-Zeta	protein kinase C, zeta	400920	722	383711	1882	
AS	PKC-Zeta	protein kinase C, zeta	400921	723	383712	1883	
AS	PKC-Zeta	protein kinase C, zeta	461106	724	426412	1884	
AS	PKC-Zeta	protein kinase C, zeta	470511	725	421350	1885	
AS	PKC-Zeta	protein kinase C, zeta	470596	726	424228	1886	

AS	PKC-Zeta	protein kinase C, zeta	470986	727	421219	1887	
AS	PKC-Zeta	protein kinase C, zeta	482686	728	4253 17	1888	
AS	PKC-Zeta	protein kinase C, zeta	496325	729	421 869	1889	
AS	PPl-cat alpha	protein phosphatase 1, catalytic subunit, alpha isozyme	3 12989	730	32603 1	1890	
AS	PPl-cat alpha	protein phosphatase 1, catalytic subunit, alpha isozyme	376745	73 1	365936	1891	
AS	PPl-cat alpha	protein phosphatase 1, catalytic subunit, alpha isozyme	45 1458	732	405603	1892	
AS	PP2a catalytic	protein phosphatase 2, catalytic subunit, alpha isozyme	481 195	733	4 18447	1893	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1H	228705	734	228705	1894	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IF	263212	735	263212	1895	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IB	282412	736	282412	1896	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IK	295908	737	295908	1897	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1M	296487	738	296487	1898	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, ID	305921	739	306682	1899	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IE	308249	740	3 1241 1	1900	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1J	309276	741	308926	1901	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IK	3 15 194	742	324761	1902	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1M	323588	743	3 19894	1903	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IN (putative)	324688	744	321761	1904	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1A	325642	745	327255	1905	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1A	325658	746	3 14850	1906	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1G	344034	747	342778	1907	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IB	345249	748	326089	1908	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1G	350803	749	264714	1909	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1J	359994	750	353088	1910	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IB	37855 1	75 1	367813	191 1	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, ID	392995	752	376720	1912	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1A	395076	753	3785 14	1913	

AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1G	395543	754	378913	1914	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IN (putative)	396734	755	379960	1915	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IF	397495	756	380632	1916	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IF	406981	757	384715	1917	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IF	407142	758	384930	1918	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IB	409432	759	387287	1919	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1M	409502	760	387046	1920	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IB	409895	761	387341	1921	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IB	419807	762	390087	1922	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IE	443 121	763	390257	1923	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1M	45735 1	764	393747	1924	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1L	497343	765	420354	1925	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1L	498165	766	417659	1926	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IK	506423	767	424155	1927	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1A	525399	768	435398	1928	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1A	528241	769	43 1453	1929	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1A	529574	770	432966	1930	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1A	53 1937	771	435575	193 1	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, IF	538191	772	439915	1932	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, 1G	544412	773	442536	1933	
AS	PP2C	protein phosphatase, Mg2+/Mn2+ dependent, ID	544712	774	4385 18	1934	
AS	Puma	BCL2 binding component 3	300880	775	300880	1935	
AS	Puma	BCL2 binding component 3	341983	776	341 155	1936	
AS	Puma	BCL2 binding component 3	439096	777	395862	1937	
AS	Puma	BCL2 binding component 3	449228	778	404503	1938	
AS	RAIDD	CASP2 and RIPKl domain containing adaptor with death domain	332896	779	327647	1939	
AS	RAIDD	CASP2 and RIPKl domain containing adaptor with death domain	541 813	780	442624	1940	

AS	RAIDD	CASP2 and RIPKl domain containing adaptor with death domain	542893	781	439068	1941	
AS	RAIDD	CASP2 and RIPKl domain containing adaptor with death domain	551065	782	448425	1942	
AS	RANK	tumor necrosis factor receptor superfamily, member 11a, NFKB activator	269485	783	269485	1943	
AS	RANK	tumor necrosis factor receptor superfamily, member 11a, NFKB activator	382790	784	372240	1944	
AS	RANKL	tumor necrosis factor (ligand) superfamily, member 11	239849	785	239849	1945	
AS	RANKL	tumor necrosis factor (ligand) superfamily, member 11	358545	786	351347	1946	
AS	RANKL	tumor necrosis factor (ligand) superfamily, member 11	398795	787	381775	1947	
AS	RANKL	tumor necrosis factor (ligand) superfamily, member 11	405262	788	384042	1948	
AS	RANKL	tumor necrosis factor (ligand) superfamily, member 11	544862	789	444913	1949	
AS	RelA (p65 NF-kappaB subunit)	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	308639	790	311508	1950	
AS	RelA (p65 NF-kappaB subunit)	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	406246	791	384273	1951	
AS	RelA (p65 NF-kappaB subunit)	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	426617	792	437980	1952	
AS	RelA (p65 NF-kappaB subunit)	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	525693	793	432537	1953	
AS	RelA (p65 NF-kappaB subunit)	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	526283	794	435290	1954	
AS	RelA (p65 NF-kappaB subunit)	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	545816	795	443700	1955	
AS	RIPKl	receptor (TNFRSF)-interacting serine-threonine kinase 1	259808	796	259808	1956	
AS	RIPKl	receptor (TNFRSF)-interacting	380409	797	369773	1957	

		serine-threonine kinase 1					
AS	RIPK1	receptor (TNFRSF)-interacting serine-threonine kinase 1	453483	798	415981	1958	
AS	RIPK1	receptor (TNFRSF)-interacting serine-threonine kinase 1	541791	799	442294	1959	
AS	Sequestosome 1 (p62)	sequestosome 1	360718	800	353944	1960	
AS	Sequestosome 1 (p62)	sequestosome 1	376929	801	366128	1961	
AS	Sequestosome 1 (p62)	sequestosome 1	389805	802	374455	1962	
AS	Sequestosome 1 (p62)	sequestosome 1	402874	803	385553	1963	
AS	Sequestosome 1 (p62)	sequestosome 1	422245	804	394534	1964	
AS	Sequestosome 1 (p62)	sequestosome 1	454378	805	408107	1965	
AS	Sequestosome 1 (p62)	sequestosome 1	514093	806	427308	1966	
AS	Shc	SHC (Src homology 2 domain containing) transforming protein 2	264554	807	264554	1967	
AS	Shc	SHC (Src homology 2 domain containing) transforming protein 1	366442	808	396162	1968	
AS	Shc	SHC (Src homology 2 domain containing) transforming protein 1	368441	809	357426	1969	
AS	Shc	SHC (Src homology 2 domain containing) transforming protein 1	368443	810	357428	1970	
AS	Shc	SHC (Src homology 2 domain containing) transforming protein 1	368445	811	357430	1971	
AS	Shc	SHC (Src homology 2 domain containing) transforming protein 1	368449	812	357434	1972	
AS	Shc	SHC (Src homology 2 domain containing) transforming protein 1	368450	813	357435	1973	
AS	Shc	SHC (Src homology 2 domain containing) transforming protein 1	368453	814	357438	1974	
AS	Shc	SHC (Src homology 2 domain containing) transforming protein 3	375830	815	364990	1975	
AS	Shc	SHC (Src homology 2 domain containing) transforming	375831	816	364991	1976	

		protein 3					
AS	She	SHC (Src homology 2 domain containing) transforming protein 3	375835	817	364995	1977	
AS	She	SHC (Src homology 2 domain containing) transforming protein 1	412170	818	398441	1978	
AS	She	SHC (Src homology 2 domain containing) transforming protein 1	414115	819	404908	1979	
AS	She	SHC (Src homology 2 domain containing) transforming protein 1	444179	820	398864	1980	
AS	She	SHC (Src homology 2 domain containing) transforming protein 1	444664	821	396333	1981	
AS	She	SHC (Src homology 2 domain containing) transforming protein 1	448116	822	401303	1982	
AS	Siah-1	seven in absentia homolog 1 (Drosophila)	356721	823	349156	1983	
AS	Siah-1	seven in absentia homolog 1 (Drosophila)	380006	824	369343	1984	
AS	Siah-1	seven in absentia homolog 1 (Drosophila)	394725	825	378214	1985	
AS	SMAC	diablo, IAP-binding mitochondrial protein	NA	826	NA	1986	
AS	Smac/Diablo	diablo, IAP-binding mitochondrial protein	267169	827	267169	1987	
AS	Smac/Diablo	diablo, IAP-binding mitochondrial protein	353548	828	320343	1988	
AS	Smac/Diablo	diablo, IAP-binding mitochondrial protein	413918	829	411638	1989	
AS	Smac/Diablo	diablo, IAP-binding mitochondrial protein	443649	830	398495	1990	
AS	Smac/Diablo	diablo, IAP-binding mitochondrial protein	464942	831	442360	1991	
AS	SODD	BCL2-associated athanogene 4	287322	832	287322	1992	
AS	SODD	BCL2-associated athanogene 4	432471	833	393298	1993	
AS	SOS	son of sevenless homolog 2 (Drosophila)	216373	834	216373	1994	
AS	SOS	son of sevenless homolog 1 (Drosophila)	263879	835	263879	1995	
AS	SOS	son of sevenless homolog 1 (Drosophila)	395038	836	378479	1996	
AS	SOS	son of sevenless homolog 1 (Drosophila)	402219	837	384675	1997	
AS	SOS	son of sevenless homolog 1 (Drosophila)	426016	838	387784	1998	
AS	SOS	son of sevenless homolog 1 (Drosophila)	428721	839	399992	1999	
AS	SOS	son of sevenless homolog 2 (Drosophila)	543680	840	445328	2000	

AS	SOS	son of sevenless homolog 1 (Drosophila)	543698	841	441 172	2001	
AS	SUMO-1	SMT3 suppressor of mif two 3 homolog 1 (S. cerevisiae)	392244	842	376075	2002	
AS	SUMO-1	SMT3 suppressor of mif two 3 homolog 1 (S. cerevisiae)	392245	843	376076	2003	
AS	SUMO-1	SMT3 suppressor of mif two 3 homolog 1 (S. cerevisiae)	392246	844	376077	2004	
AS	SUMO-1	SMT3 suppressor of mif two 3 homolog 1 (S. cerevisiae)	409205	845	386267	2005	
AS	SUMO-1	SMT3 suppressor of mif two 3 homolog 1 (S. cerevisiae)	409498	846	386472	2006	
AS	Survivin	baculoviral IAP repeat containing 5	301633	847	301633	2007	
AS	Survivin	baculoviral IAP repeat containing 5	35005 1	848	3241 80	2008	
AS	Survivin	baculoviral IAP repeat containing 5	374948	849	364086	2009	
AS	Survivin	baculoviral IAP repeat containing 5	432014	850	389088	2010	
AS	TACI	tumor necrosis factor receptor superfamily, member 13B	261652	85 1	261652	201 1	
AS	TACI	tumor necrosis factor receptor superfamily, member 13B	437538	852	413453	2012	
AS	tBid	tBID	NA		NA	2013	
AS	TL1A	tumor necrosis factor (ligand) superfamily, member 15	374044	853	363 156	2014	
AS	TL1A	tumor necrosis factor (ligand) superfamily, member 15	374045	854	363 157	2015	
AS	TNF-alpha	tumor necrosis factor	376122	855	365290	2016	
AS	TNF-alpha	tumor necrosis factor	383496	856	372988	2017	
AS	TNF-alpha	tumor necrosis factor	412275	857	392858	201 8	
AS	TNF-alpha	tumor necrosis factor	420425	858	410668	2019	
AS	TNF-alpha	tumor necrosis factor	443707	859	389492	2020	
AS	TNF-alpha	tumor necrosis factor	445232	860	389265	2021	
AS	TNF-alpha	tumor necrosis factor	448781	861	389490	2022	
AS	TNF-alpha	tumor necrosis factor	449264	862	398698	2023	
AS	TNF-R1	tumor necrosis factor receptor superfamily, member 1A	162749	863	162749	2024	
AS	TNF-R1	tumor necrosis factor receptor superfamily, member 1A	366159	864	380389	2025	
AS	TNF-R2	tumor necrosis factor receptor superfamily, member IB	376259	865	365435	2026	
AS	TNF-R2	tumor necrosis factor receptor superfamily, member IB	376259	866	365435	2027	2491
AS	TNF-R2	tumor necrosis factor receptor	400863	867	383660	2028	

		superfamily, member IB					
AS	TNF-R2	tumor necrosis factor receptor superfamily, member IB	536782	868	440425	2029	
AS	TRADD	TNFRSFlA-associated via death domain	345057	869	341268	2030	
AS	TRAF2	TNF receptor-associated factor 2	247668	870	247668	203 1	
AS	TRAF2	TNF receptor-associated factor 2	359662	871	352685	2032	
AS	TRAF2	TNF receptor-associated factor 2	371645	872	360708	2033	
AS	TRAF2	TNF receptor-associated factor 2	414589	873	397653	2034	
AS	TRAF2	TNF receptor-associated factor 2	419057	874	405860	2035	
AS	TRAF2	TNF receptor-associated factor 2	429509	875	406524	2036	
AS	TRAF2	TNF receptor-associated factor 2	432785	876	400061	2037	
AS	TRAF2	TNF receptor-associated factor 2	536468	877	446414	2038	
AS	TRAF3	TNF receptor-associated factor 3	347662	878	328003	2039	
AS	TRAF3	TNF receptor-associated factor 3	35 1691	879	332468	2040	
AS	TRAF3	TNF receptor-associated factor 3	392745	880	376500	2041	
AS	TRAF3	TNF receptor-associated factor 3	539721	881	445998	2042	
AS	TRAF3	TNF receptor-associated factor 3	560371	882	454207	2043	
AS	TRAF3	TNF receptor-associated factor 3	560463	883	453623	2044	
AS	TRAF5	TNF receptor-associated factor 5	261464	884	261464	2045	
AS	TRAF5	TNF receptor-associated factor 5	3361 84	885	336825	2046	
AS	TRAF5	TNF receptor-associated factor 5	367004	886	355971	2047	
AS	TRAF5	TNF receptor-associated factor 5	427925	887	389891	2048	
AS	TRAF6	TNF receptor-associated factor 6	348124	888	337853	2049	
AS	TRAF6	TNF receptor-associated factor 6	526995	889	433623	2050	
AS	TrkA	neurotrophic tyrosine kinase, receptor, type 1	368196	890	357179	2051	
AS	TrkA	neurotrophic tyrosine kinase, receptor, type 1	392302	891	376120	2052	
AS	TrkA	neurotrophic tyrosine kinase, receptor, type 1	524377	892	43 141 8	2053	
AS	TWEAK (TNFSF 12)	tumor necrosis factor (ligand) superfamily, member 12	293825	893	293825	2054	

AS	TWEAK (TNFSF 12)	tumor necrosis factor (ligand) superfamily, member 12	557233	894	451451	2055	
AS	VDAC 1	voltage-dependent anion channel 1	265333	895	265333	2056	
AS	VDAC 1	voltage-dependent anion channel 1	395044	896	378484	2057	
AS	VDAC 1	voltage-dependent anion channel 1	395047	897	378487	2058	
AS	VDAC 2	voltage-dependent anion channel 2	298468	898	298468	2059	
AS	VDAC 2	voltage-dependent anion channel 2	313132	899	361635	2060	
AS	VDAC 2	voltage-dependent anion channel 2	332211	900	361686	2061	
AS	VDAC 2	voltage-dependent anion channel 2	344036	901	344876	2062	
AS	VDAC 2	voltage-dependent anion channel 2	413289	902	389551	2063	
AS	VDAC 2	voltage-dependent anion channel 2	447677	903	401492	2064	
AS	VDAC 2	voltage-dependent anion channel 2	535553	904	445901	2065	
AS	VDAC 2	voltage-dependent anion channel 2	543351	905	443092	2066	
AS	XIAP	X-linked inhibitor of apoptosis	355640	906	347858	2067	
AS	XIAP	X-linked inhibitor of apoptosis	371199	907	360242	2068	
AS	XIAP	X-linked inhibitor of apoptosis	430625	908	400637	2069	
AS	XIAP	X-linked inhibitor of apoptosis	434753	909	395230	2070	
AS	XIAP	X-linked inhibitor of apoptosis	NA	910	NA	2071	
CC/S	ATM	ataxia telangiectasia mutated	278616	911	278616	2072	
CC/S	ATM	ataxia telangiectasia mutated	389511	912	374162	2073	
CC/S	ATM	ataxia telangiectasia mutated	452508	913	388058	2074	
CC/S	ATM	ataxia telangiectasia mutated	532931	914	432318	2075	
CC/S	ATR	ataxia telangiectasia and Rad3 related	350721	915	343741	2076	
CC/S	ATR	ataxia telangiectasia and Rad3 related	383101	916	372581	2077	
CC/S	ATRIP	ATR interacting protein	320211	917	323099	2078	
CC/S	ATRIP	ATR interacting protein	346691	918	302338	2079	
CC/S	ATRIP	ATR interacting protein	357105	919	349620	2080	
CC/S	ATRIP	ATR interacting protein	412052	920	400930	2081	
CC/S	ATRIP	ATR interacting protein	421175	921	406664	2082	
CC/S	Bard1	BRCA1 associated RING domain 1	260947	922	260947	2083	
CC/S	Bard1	BRCA1 associated RING domain 1	449967	923	406752	2084	
CC/S	BLM	Bloom syndrome, RecQ helicase-like	355112	924	347232	2085	
CC/S	BLM	Bloom syndrome, RecQ	536925	925	442330	2086	

		helicase-like					
CC/S	BLM	Bloom syndrome, RecQ helicase-like	543977	926	439075	2087	
CC/S	Brcal	breast cancer 1, early onset	309486	927	310938	2088	
CC/S	Brcal	breast cancer 1, early onset	346315	928	246907	2089	
CC/S	Brcal	breast cancer 1, early onset	351666	929	338007	2090	
CC/S	Brcal	breast cancer 1, early onset	352993	930	312236	2091	
CC/S	Brcal	breast cancer 1, early onset	354071	931	326002	2092	
CC/S	Brcal	breast cancer 1, early onset	357654	932	350283	2093	
CC/S	Brcal	breast cancer 1, early onset	393691	933	377294	2094	
CC/S	Brcal	breast cancer 1, early onset	412061	934	397145	2095	
CC/S	Brcal	breast cancer 1, early onset	461221	935	418548	2096	
CC/S	Brcal	breast cancer 1, early onset	461798	936	417988	2097	
CC/S	Brcal	breast cancer 1, early onset	468300	937	417148	2098	
CC/S	Brcal	breast cancer 1, early onset	470026	938	419274	2099	
CC/S	Brcal	breast cancer 1, early onset	471181	939	418960	2100	
CC/S	Brcal	breast cancer 1, early onset	476777	940	417554	2101	
CC/S	Brcal	breast cancer 1, early onset	477152	941	419988	2102	
CC/S	Brcal	breast cancer 1, early onset	478531	942	420412	2103	
CC/S	Brcal	breast cancer 1, early onset	484087	943	419481	2104	
CC/S	Brcal	breast cancer 1, early onset	489037	944	420781	2105	
CC/S	Brcal	breast cancer 1, early onset	491747	945	420705	2106	
CC/S	Brcal	breast cancer 1, early onset	492859	946	420253	2107	
CC/S	Brcal	breast cancer 1, early onset	493795	947	418775	2108	
CC/S	Brcal	breast cancer 1, early onset	493919	948	418819	2109	
CC/S	Brcal	breast cancer 1, early onset	494123	949	419103	2110	
CC/S	Brcal	breast cancer 1, early onset	497488	950	418986	2111	
CC/S	c-Abl	c-abl oncogene 1, non-receptor tyrosine kinase	318560	951	323315	2112	
CC/S	c-Abl	c-abl oncogene 1, non-receptor tyrosine kinase	372348	952	361423	2113	
CC/S	c-Abl	c-abl oncogene 1, non-receptor tyrosine kinase	393293	953	376971	2114	
CC/S	c-Abl	c-abl oncogene 1, non-receptor tyrosine kinase	438426	954	407756	2115	
CC/S	c-Abl	c-abl oncogene 1, non-receptor tyrosine kinase	444970	955	400412	2116	
CC/S	CDC25 A	cell division cycle 25 homolog A (S. pombe)	302506	956	303706	2117	
CC/S	CDC25 A	cell division cycle 25 homolog A (S. pombe)	351231	957	343166	2118	
CC/S	CDC25 A	cell division cycle 25 homolog A (S. pombe)	437972	958	404285	2119	
CC/S	CDC25 B	cell division cycle 25 homolog B (S. pombe)	245960	959	245960	2120	
CC/S	CDC25 B	cell division cycle 25 homolog B (S. pombe)	340833	960	339170	2121	

CC/S	CDC25 B	cell division cycle 25 homolog B (S. pombe)	344256	961	339125	2122	
CC/S	CDC25 B	cell division cycle 25 homolog B (S. pombe)	379598	962	36891 8	2123	
CC/S	CDC25 B	cell division cycle 25 homolog B (S. pombe)	439880	963	405972	2124	
CC/S	CDC25 C	cell division cycle 25 homolog C (S. pombe)	323760	964	321656	2125	
CC/S	CDC25 C	cell division cycle 25 homolog C (S. pombe)	348983	965	345205	2126	
CC/S	CDC25 C	cell division cycle 25 homolog C (S. pombe)	356505	966	348898	2127	
CC/S	CDC25 C	cell division cycle 25 homolog C (S. pombe)	357274	967	349821	2128	
CC/S	CDC25 C	cell division cycle 25 homolog C (S. pombe)	415 130	968	39263 1	2129	
CC/S	CDC25 C	cell division cycle 25 homolog C (S. pombe)	503022	969	42725 1	2130	
CC/S	CDC25 C	cell division cycle 25 homolog C (S. pombe)	5 13970	970	424795	213 1	
CC/S	CDC25 C	cell division cycle 25 homolog C (S. pombe)	534892	971	443 196	2132	
CC/S	CDK2	cyclin-dependent kinase 2	266970	972	266970	2133	
CC/S	CDK2	cyclin-dependent kinase 2	354056	973	243067	2134	
CC/S	CDK4	cyclin-dependent kinase 4	257904	974	257904	2135	
CC/S	CDK4	cyclin-dependent kinase 4	3 12990	975	3 16889	2136	
CC/S	CDK4	cyclin-dependent kinase 4	540325	976	439076	2137	
CC/S	CDK4	cyclin-dependent kinase 4	552254	977	449179	2138	
CC/S	CDK4	cyclin-dependent kinase 4	552388	978	448963	2139	
CC/S	CDK4	cyclin-dependent kinase 4	552862	979	446763	2140	
CC/S	CDK6	cyclin-dependent kinase 6	265734	980	265734	2141	
CC/S	CDK6	cyclin-dependent kinase 6	424848	981	397087	2142	
CC/S	Chkl	checkpoint kinase 1	278916	982	278916	2143	
CC/S	Chkl	checkpoint kinase 1	428830	983	412504	2144	
CC/S	Chkl	checkpoint kinase 1	438015	984	388648	2145	
CC/S	Chkl	checkpoint kinase 1	524737	985	432890	2146	
CC/S	Chkl	checkpoint kinase 1	525396	986	434141	2147	
CC/S	Chkl	checkpoint kinase 1	526937	987	43 1815	2148	
CC/S	Chkl	checkpoint kinase 1	527013	988	43 1525	2149	
CC/S	Chkl	checkpoint kinase 1	534070	989	435371	2150	
CC/S	Chkl	checkpoint kinase 1	534685	990	432470	215 1	
CC/S	Chkl	checkpoint kinase 1	544373	991	4423 17	2152	
CC/S	Chk2	checkpoint kinase 2	328354	992	329178	2153	
CC/S	Chk2	checkpoint kinase 2	348295	993	329012	2154	
CC/S	Chk2	checkpoint kinase 2	382563	994	372003	2155	
CC/S	Chk2	checkpoint kinase 2	382565	995	372006	2156	
CC/S	Chk2	checkpoint kinase 2	382566	996	372007	2157	

CC/S	Chk2	checkpoint kinase 2	382578	997	372021	2158	
CC/S	Chk2	checkpoint kinase 2	382580	998	372023	2159	
CC/S	Chk2	checkpoint kinase 2	40273 1	999	384835	2160	
CC/S	Chk2	checkpoint kinase 2	403642	1000	384919	2161	
CC/S	Chk2	checkpoint kinase 2	404276	1001	385747	2162	
CC/S	Chk2	checkpoint kinase 2	405598	1002	386087	2163	
CC/S	Chk2	checkpoint kinase 2	544772	1003	442458	2164	
CC/S	Claspin	claspin	25 1195	1004	25 1195	2165	
CC/S	Claspin	claspin	3 18121	1005	3 12995	2166	
CC/S	Claspin	claspin	373220	1006	3623 17	2167	
CC/S	Claspin	claspin	544356	1007	442335	2168	
CC/S	Cyclin A	cyclin A2	274026	1008	274026	2169	
CC/S	Cyclin B	cyclin B1	256442	1009	256442	2170	
CC/S	Cyclin B	cyclin B3	276014	1010	276014	2171	
CC/S	Cyclin B	cyclin B2	288207	101 1	288207	2172	
CC/S	Cyclin B	cyclin B3	348603	1012	338682	2173	
CC/S	Cyclin B	cyclin B3	376038	1013	365206	2174	
CC/S	Cyclin B	cyclin B3	376042	1014	365210	2175	
CC/S	Cyclin B	cyclin B3	396540	1015	379790	2176	
CC/S	Cyclin B	cyclin B1	505500	1016	424588	2177	
CC/S	Cyclin B	cyclin B1	506572	1017	423387	2178	
CC/S	Cyclin D	cyclin D1	227507	1018	227507	2179	
CC/S	Cyclin D	cyclin D2	261254	1019	261254	2 180	
CC/S	Cyclin D	cyclin D3	372987	1020	362078	2 18 1	
CC/S	Cyclin D	cyclin D3	372988	1021	362079	2 182	
CC/S	Cyclin D	cyclin D3	372991	1022	362082	2 183	
CC/S	Cyclin D	cyclin D3	414200	1023	397545	2 184	
CC/S	Cyclin D	cyclin D3	415497	1024	401595	2 185	
CC/S	Cyclin D	cyclin D3	505064	1025	425830	2 186	
CC/S	Cyclin D	cyclin D3	5 11642	1026	426212	2 187	
CC/S	Cyclin D	cyclin D1	542897	1027	441 863	2 188	
CC/S	Cyclin E	cyclin E1	262643	1028	262643	2 189	
CC/S	Cyclin E	cyclin E2	308108	1029	3091 8 1	2190	
CC/S	Cyclin E	cyclin E1	357943	1030	350625	2191	
CC/S	Cyclin E	cyclin E2	396133	103 1	379437	2192	
CC/S	Cyclin E	cyclin E1	444983	1032	410179	2193	
CC/S	Cyclin E	cyclin E2	520509	1033	429089	2194	
CC/S	Cyclin E	cyclin E2	542725	1034	445726	2195	
CC/S	DNA-PK	protein kinase, DNA-activated, catalytic polypeptide	3 14191	1035	3 13420	2196	
CC/S	DNA-PK	protein kinase, DNA-activated, catalytic polypeptide	338368	1036	345 182	2197	

CC/S	E2F1/2/3/4/5/6	E2F transcription factor 5, pl30-binding	2561 17	1037	2561 17	2198	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 6	307236	1038	302159	2199	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 1	343380	1039	345571	2200	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 3	346618	1040	262904	2201	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 2	361729	1041	355249	2202	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 6	362009	1042	355036	2203	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 3	378646	1043	367914	2204	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 4, pl07/pl30-binding	379378	1044	368686	2205	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 6	381525	1045	370936	2206	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 5, pl30-binding	416274	1046	398124	2207	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 5, pl30-binding	4 18930	1047	4143 12	2208	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 5, pl30-binding	5 17476	1048	429120	2209	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 5, pl30-binding	5 18234	1049	429669	2210	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 3	535432	1050	44341 8	221 1	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 6	542100	105 1	4463 15	2212	
CC/S	E2F1/2/3/4/5/6	E2F transcription factor 6	546212	1052	438864	2213	
CC/S	FANCD2	Fanconi anemia, complementation group D2	287647	1053	287647	2214	
CC/S	FANCD2	Fanconi anemia, complementation group D2	383806	1054	3733 17	2215	
CC/S	FANCD2	Fanconi anemia, complementation group D2	383807	1055	3733 18	2216	
CC/S	FANCD2	Fanconi anemia, complementation group D2	419585	1056	398754	2217	
CC/S	FANCL	Fanconi anemia, complementation group L	233741	1057	233741	2218	
CC/S	FANCL	Fanconi anemia, complementation group L	540646	1058	44143 1	2219	
CC/S	GADD45 alpha	growth arrest and DNA-damage-inducible, alpha	370986	1059	360025	2220	
CC/S	GADD45 beta	growth arrest and DNA-damage-inducible, beta	21563 1	1060	21563 1	2221	
CC/S	GADD45 beta	growth arrest and DNA-damage-inducible, alpha	370985	1061	360024	2222	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	258148	1062	258148	2223	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	258149	1063	258149	2224	

CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	299252	1064	299252	2225	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	311420	1065	310742	2226	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	311440	1066	311302	2227	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	348801	1067	335096	2228	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	350057	1068	266624	2229	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	356290	1069	348637	2230	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	358483	1070	351270	2231	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	360430	1071	353611	2232	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	393410	1072	377062	2233	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	393412	1073	377064	2234	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	393413	1074	377065	2235	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	393415	1075	377067	2236	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	428863	1076	410694	2237	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	462284	1077	417281	2238	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	517852	1078	430257	2239	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	539479	1079	444430	2240	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	540827	1080	440932	2241	
CC/S	MDM2	Mdm2 p53 binding protein homolog (mouse)	544648	1081	443274	2242	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	376405	1082	365587	2243	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	376406	1083	365588	2244	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	383566	1084	373060	2245	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	412395	1085	392833	2246	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	413973	1086	408831	2247	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	416368	1087	410383	2248	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	416571	1088	400979	2249	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	417033	1089	408962	2250	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	417228	1090	400305	2251	

CC/S	NFBD1	mediator of DNA-damage checkpoint 1	419172	1091	398474	2252	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	419675	1092	397642	2253	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	420019	1093	396484	2254	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	420320	1094	4165 11	2255	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	422104	1095	390375	2256	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	422195	1096	407703	2257	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	422266	1097	4 113 10	2258	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	423726	1098	391230	2259	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	424437	1099	39815 1	2260	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	424507	1100	388355	2261	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	424638	1101	394074	2262	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	425029	1102	397126	2263	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	425072	1103	396989	2264	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	425790	1104	397021	2265	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	427406	1105	387429	2266	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	429610	1106	406850	2267	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	430358	1107	414163	2268	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	43 1441	1108	392784	2269	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	432998	1109	405991	2270	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	435664	1110	4043 18	2271	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	435797	1111	400677	2272	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	437759	1112	387743	2273	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	438165	1113	387706	2274	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	440369	1114	415212	2275	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	441397	1115	390489	2276	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	444412	1116	413610	2277	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	445 130	1117	396124	2278	

CC/S	NFBD1	mediator of DNA-damage checkpoint 1	445764	1118	393886	2279	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	447192	1119	405806	2280	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	447640	1120	396389	2281	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	448895	1121	396121	2282	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	449153	1122	409167	2283	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	450033	1123	390040	2284	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	452213	1124	404936	2285	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	455729	1125	404954	2286	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	456589	1126	405350	2287	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	546487	1127	448679	2288	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	546539	1128	448232	2289	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	547047	1129	449059	2290	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	547353	1130	447883	2291	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	547681	1131	447851	2292	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	547700	1132	449083	2293	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	547874	1133	447682	2294	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	548103	1134	449499	2295	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	54812	1135	448434	2296	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	548248	1136	448080	2297	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	548433	1137	449971	2298	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	548542	1138	446597	2299	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	548805	1139	446924	2300	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	548827	1140	449201	2301	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	548893	1141	447943	2302	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	548947	1142	44771	2303	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	549228	1143	4475 17	2304	
CC/S	NFBD1	mediator of DNA-damage checkpoint 1	549382	1144	449177	2305	

CC/S	NFBD1	mediator of DNA-damage checkpoint_1	549428	1145	447038	2306	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	549771	1146	448812	2307	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	550004	1147	447084	2308	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	5501 10	1148	446980	2309	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	550210	1149	447697	2310	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	550408	1150	447136	2311	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	550500	1151	450002	2312	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	550688	1152	448066	2313	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	551204	1153	447799	2314	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	551267	1154	450198	2315	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	551460	1155	449274	2316	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	551554	1156	448538	2317	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	551621	1157	448285	2318	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	551740	1158	450037	2319	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	552263	1159	447069	2320	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	552349	1160	449892	2321	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	552474	1161	447771	2322	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	552522	1162	449936	2323	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	552776	1163	447825	2324	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	553047	1164	447247	2325	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	553048	1165	447787	2326	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	553 130	1166	446809	2327	
CC/S	NFBD1	mediator of DNA-damage checkpoint_1	553 196	1167	449586	2328	
CC/S	Nibrin	nibrin	265433	1168	265433	2329	
CC/S	Nibrin	nibrin	452387	1169	445213	2330	
CC/S	pl07	retinoblastoma-like 1 (pi 07)	344359	1170	343646	2331	
CC/S	pl07	retinoblastoma-like 1 (pi 07)	373664	1171	362768	2332	
CC/S	pl30	retinoblastoma-like 2 (pi 30)	262133	1172	262133	2333	
CC/S	pl30	retinoblastoma-like 2 (pi 30)	379935	1173	369267	2334	

CC/S	pl30	retinoblastoma-like 2 (pi 30)	544405	1174	443744	2335	
CC/S	pl30	retinoblastoma-like 2 (pi 30)	544545	1175	444685	2336	
CC/S	p21	P21	NA	1176	NA	2337	
CC/S	PCNA	proliferating cell nuclear antigen	379143	1177	368438	2338	
CC/S	PCNA	proliferating cell nuclear antigen	379160	1178	368458	2339	
CC/S	RAD9	RAD9 homolog A (S. pombe)	307980	1179	311360	2340	
CC/S	Rb protein	retinoblastoma 1	267163	1180	267163	2341	
CC/S	Rb protein	retinoblastoma 1	467505	1181	434702	2342	
CC/S	Rb protein	retinoblastoma 1	542917	1182	437642	2343	
CC/S	SMC1	structural maintenance of chromosomes 1A	322213	1183	323421	2344	
CC/S	SMC1	structural maintenance of chromosomes 1A	340213	1184	344906	2345	
CC/S	SMC1	structural maintenance of chromosomes 1A	375340	1185	364489	2346	
CC/S	SMC1	structural maintenance of chromosomes 1A	428014	1186	413509	2347	
CC/S	USP1	ubiquitin specific peptidase 1	339950	1187	343526	2348	
CC/S	USP1	ubiquitin specific peptidase 1	371146	1188	360188	2349	
CC/S	USP1	ubiquitin specific peptidase 1	452143	1189	403662	2350	
M	4EBP-1	eukaryotic translation initiation factor 4E binding protein 1	338825	1190	340691	2351	
M	ARNT	aryl hydrocarbon receptor nuclear translocator	354396	1191	346372	2352	
M	ARNT	aryl hydrocarbon receptor nuclear translocator	358595	1192	351407	2353	
M	ARNT	aryl hydrocarbon receptor nuclear translocator	368975	1193	357971	2354	
M	ARNT	aryl hydrocarbon receptor nuclear translocator	394700	1194	378190	2355	
M	ARNT	aryl hydrocarbon receptor nuclear translocator	471844	1195	425899	2356	
M	ARNT	aryl hydrocarbon receptor nuclear translocator	505755	1196	427571	2357	
M	ARNT	aryl hydrocarbon receptor nuclear translocator	515192	1197	423851	2358	
M	CAIX	carbonic anhydrase IX	378357	1198	367608	2359	
M	CAIX	carbonic anhydrase IX	544074	1199	438541	2360	
M	CBP	CREB binding protein	262367	1200	262367	2361	
M	CBP	CREB binding protein	323508	1201	323550	2362	
M	CBP	CREB binding protein	382070	1202	371502	2363	
M	CBP	CREB binding protein	543883	1203	441978	2364	
M	CITED 1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	246139	1204	246139	2365	

M	CITED 1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	373619	1205	362721	2366	
M	CITED 1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	417400	1206	414781	2367	
M	CITED 1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	427412	1207	391407	2368	
M	CITED 1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	429794	1208	407496	2369	
M	CITED 1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	431381	1209	388548	2370	
M	CITED 1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	445983	1210	403274	2371	
M	CITED 1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	450875	1211	405765	2372	
M	CITED 1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	453707	1212	401764	2373	
M	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	367651	1213	356623	2374	
M	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	392312	1214	376126	2375	
M	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	536159	1215	442831	2376	
M	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	537332	1216	444198	2377	
M	CITED4	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 4	NA	1217	NA	2378	
M	CITED4	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain,	372638	1218	361721	2379	

		4 (CBP/p300 interacting transactivator with ED-rich tail)					
M	COMMD1	copper metabolism (Murr1) domain containing 1	311832	1219	308236	2380	
M	COMMD1	copper metabolism (Murr1) domain containing 1	427417	1220	413207	2381	
M	COMMD1	copper metabolism (Murr1) domain containing 1	444166	1221	410050	2382	
M	COMMD1	copper metabolism (Murr1) domain containing 1	458337	1222	401236	2383	
M	COMMD1	copper metabolism (Murr1) domain containing 1	538736	1223	438961	2384	
M	CREB	cAMP responsive element binding protein 1	236996	1224	236996	2385	
M	CREB	cAMP responsive element binding protein 1	353267	1225	236995	2386	
M	CREB	cAMP responsive element binding protein 3	353704	1226	342136	2387	
M	CREB	cAMP responsive element binding protein 1	374397	1227	363518	2388	
M	CREB	cAMP responsive element binding protein 1	430624	1228	405539	2389	
M	CREB	cAMP responsive element binding protein 1	432329	1229	387699	2390	
M	CREB	cAMP responsive element binding protein 1	445803	1230	407227	2391	
M	CREB	cAMP responsive element binding protein 1	452474	1231	392428	2392	
M	CREB	cAMP responsive element binding protein 1	536726	1232	445892	2393	
M	CREB	cAMP responsive element binding protein 1	539789	1233	440809	2394	
M	eIF4E	eukaryotic translation initiation factor 4E	280892	1234	280892	2395	
M	eIF4E	eukaryotic translation initiation factor 4E	450253	1235	389624	2396	
M	HIF3-alpha	hypoxia inducible factor 3, alpha subunit	244302	1236	244302	2397	
M	HIF3-alpha	hypoxia inducible factor 3, alpha subunit	291300	1237	291300	2398	
M	FIH	hypoxia inducible factor 1, alpha subunit inhibitor (factor inhibiting HIF)	299163	1238	299163	2399	
M	HIF3-alpha)	hypoxia inducible factor 3, alpha subunit	300862	1239	300862	2400	
M	HIF3-alpha	hypoxia inducible factor 3, alpha subunit	339613	1240	341877	2401	
M	HIF3-alpha	hypoxia inducible factor 3, alpha subunit	377670	1241	366898	2402	
M	HIF3-alpha	hypoxia inducible factor 3, alpha subunit	414707	1242	412808	2403	
M	HIF3-alpha	hypoxia inducible factor 3, alpha subunit	420102	1243	407771	2404	

M	FIH (factor inhibiting HIF)	hypoxia inducible factor 1, alpha subunit inhibitor	442724	1244	399734	2405	
M	HIF3-alpha	hypoxia inducible factor 3, alpha subunit	457771	1245	408008	2406	
M	HIF3-alpha	hypoxia inducible factor 3, alpha subunit	457865	1246	394052	2407	
M	HIF3-alpha	hypoxia inducible factor 3, alpha subunit	475432	1247	432578	2408	
M	FIH (factor inhibiting HIF)	hypoxia inducible factor 1, alpha subunit inhibitor	533589	1248	433360	2409	
M	Grb2	growth factor receptor-bound protein 2	316615	1249	317360	2410	
M	Grb2	growth factor receptor-bound protein 2	316804	1250	339007	2411	
M	Grb2	growth factor receptor-bound protein 2	392562	1251	376345	2412	
M	Grb2	growth factor receptor-bound protein 2	392564	1252	376347	2413	
M	HNF4alpha	hepatocyte nuclear factor 4, alpha	316099	1253	312987	2414	
M	HNF4alpha	hepatocyte nuclear factor 4, alpha	316673	1254	315180	2415	
M	HNF4alpha	hepatocyte nuclear factor 4, alpha	338692	1255	343807	2416	
M	HNF4alpha	hepatocyte nuclear factor 4, alpha	415691	1256	412111	2417	
M	HNF4alpha	hepatocyte nuclear factor 4, alpha	443598	1257	410911	2418	
M	HNF4alpha	hepatocyte nuclear factor 4, alpha	457232	1258	396216	2419	
M	HNF4alpha2	Homo sapiens hepatocyte nuclear factor 4, alpha (HNF4A), transcript variant 2, mRNA	NA	1259	NA	2420	
M	IBP3	insulin-like growth factor binding protein 3	275521	1260	275521	2421	
M	IBP3	insulin-like growth factor binding protein 3	381083	1261	370473	2422	
M	IBP3	insulin-like growth factor binding protein 3	381086	1262	370476	2423	
M	IBP3	insulin-like growth factor binding protein 3	417621	1263	399116	2424	
M	IBP3	insulin-like growth factor binding protein 3	428530	1264	390298	2425	
M	IBP3	insulin-like growth factor binding protein 3	433047	1265	404461	2426	
M	IBP3	insulin-like growth factor binding protein 3	438491	1266	393740	2427	
M	IBP3	insulin-like growth factor binding protein 3	442142	1267	392472	2428	

M	IBP3	insulin-like growth factor binding protein 3	545032	1268	439999	2429	
M	JAB1	COP9 constitutive photomorphogenic homolog subunit 5 (Arabidopsis)	357849	1269	350512	2430	
M	MNK1	MAP kinase interacting serine/threonine kinase 1	341183	1270	339573	2431	
M	MNK1	MAP kinase interacting serine/threonine kinase 1	371944	1271	361012	2432	
M	MNK1	MAP kinase interacting serine/threonine kinase 1	371945	1272	361013	2433	
M	MNK1	MAP kinase interacting serine/threonine kinase 1	371946	1273	361014	2434	
M	MNK1	MAP kinase interacting serine/threonine kinase 1	428112	1274	411135	2435	
M	MNK1	MAP kinase interacting serine/threonine kinase 1	496619	1275	436709	2436	
M	MNK1	MAP kinase interacting serine/threonine kinase 1	545730	1276	440974	2437	
M	MNK2	MAP kinase interacting serine/threonine kinase 2	250896	1277	250896	2438	
M	MNK2	MAP kinase interacting serine/threonine kinase 2	309340	1278	309485	2439	
M	MNK2	MAP kinase interacting serine/threonine kinase 2	541165	1279	438904	2440	
M	MNK2	MAP kinase interacting serine/threonine kinase 2	545627	1280	441245	2441	
M	p15(INK 4A)	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	276925	1281	276925	2442	
M	p15(INK 4A)	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	380142	1282	369487	2443	
M	p300	E1A binding protein p300	263253	1283	263253	2444	
M	Per1	period homolog 1 (Drosophila)	317276	1284	314420	2445	
M	Per1	period homolog 1 (Drosophila)	354903	1285	346979	2446	
M	RPS6	ribosomal protein S6	315377	1286	369743	2447	
M	RPS6	ribosomal protein S6	380381	1287	369741	2448	
M	RPS6	ribosomal protein S6	380384	1288	369745	2449	
M	RPS6	ribosomal protein S6	380394	1289	369757	2450	
M	SHARP 1	basic helix-loop-helix family, member e41	NA	1290	NA	2451	
M	SHARP 1 (BHLH E41)	basic helix-loop-helix family, member e41	242728	1291	242728	2452	
M	SHARP 1 (BHLH E41)	basic helix-loop-helix family, member e41	540731	1292	437369	2453	
M	SRC1	nuclear receptor coactivator 1	288599	1293	288599	2454	
M	SRC1	nuclear receptor coactivator 1	348332	1294	320940	2455	

M	SRC1	nuclear receptor coactivator 1	395856	1295	379197	2456	
M	SRC1	nuclear receptor coactivator 1	405141	1296	385097	2457	
M	SRC1	nuclear receptor coactivator 1	406961	1297	385216	2458	
M	SRC1	nuclear receptor coactivator 1	538539	1298	444039	2459	
M	tuberin	tuberous sclerosis 2	219476	1299	219476	2460	
M	tuberin	tuberous sclerosis 2	350773	1300	344383	2461	
M	tuberin	tuberous sclerosis 2	353929	1301	248099	2462	
M	tuberin	tuberous sclerosis 2	382538	1302	371978	2463	
M	tuberin	tuberous sclerosis 2	401874	1303	384468	2464	
M	tuberin	tuberous sclerosis 2	439673	1304	399232	2465	
	AIFSH	apoptosis-inducing factor, short	NA	1305	NA	2466	
	Angiopo ietinl	Angiopoietin 1	NA	1306	NA	2467	2492
	BMP2 CO	BMP2 CO	NA	1307	NA	2468	2493
	c-MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	NA	1308	NA	2469	
	COMM D1	COMMDl	NA	1309	NA		
	COMM DI NES deleted	COMMDl with nuclear export seqences deleted	NA		NA	2470	
	COMM D1 NESl deleted andNLS added	COMMDl with nuclear export sequences deleted and nuclear localization signals added	NA		NA	2471	
	COMM D1 SV40 NLS	COMMDl with SV40 and nuclear localization signals	NA		NA	2472	
	COMM Dlwt	COMMDl wild-type	NA		NA	2473	
	GLUT1	solute carrier family 2 (facilitated glucose transporter), member 1	NA	1310	NA	2474	
	Granuly sin FL15	Granulysin FL15	NA	1311	NA	2475	
	Granuly sinNS9	Granulysin NS9	NA		NA	2476	2494
	Granuly sin S9	Granulysin S9	NA		NA	2477	2495
	HIF1 a	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	NA	1312	NA	2478	
	IL15	interleukin 15	NA	1313	NA	2479	
	KGF	fibroblast growth factor 7,	NA	1314	NA	2480	

		precursor; mature is 32-194					
	MCT4	solute carrier family 16, member 4 (monocarboxylic acid transporter 5)	NA	1315	NA	2481	2496
	MYC inhibitor D	MYC inhibitor D (OMOMyc)	NA	1316	NA	2482	
	MYC inhibitor D_90	MYC inhibitor D_90 (OmoMyc_90)	NA		NA	2483	
	C.A. caspase 3_cleavable	Constitutively active (C.A.) caspase 3 cleavable (RevCasp3_Cleavable)	NA	1317	NA	2484	
	C.A. caspase 3_uncleavable	Constitutively active (C.A.) caspase 3 uncleavable (RevCasp3_UnCleavable)	NA	1318	NA	2485	
	C.A. caspase 6	Constitutively active (C.A.) caspase 6 (RevCasp6)	NA	1319	NA	2486	
	SIAhl	siah E3 ubiquitin protein ligase 1	NA	1320	NA	2487	
	HSVl-tk	Herpes simplex virus 1-thymidine kinase					

[00299] Shown in Table 7, are familiar cancer syndromes, tumor suppressor genes, function of the tumor suppressor gene, chromosomal location, and tumor type observed. Signal-sensor polynucleotides of the present invention can be designed as a therapeutic for any of those listed in the table.

Table 7. Familial Cancer Syndrome Targets

Familial Cancer Syndrome	Tumor Suppressor Gene	Function	Chromosomal Location	Tumor Types Observed
Li-Fraumeni Syndrome	P53	cell cycle regulation, apoptosis	17p13.1	brain tumors, sarcomas, leukemia, breast cancer
Familial Retinoblastoma	RB1	cell cycle regulation	13q14.1-q14.2	retinoblastoma, osteogenic sarcoma
Wilms Tumor	WT1	transcriptional regulation	11p13	pediatric kidney cancer, most common form of childhood solid tumor
Neurofibromatosis Type 1	NF1	catalysis of RAS inactivation	17q11.2	neurofibromas, sarcomas, gliomas

Neurofibromato¬ sis Type 2	NF2	linkage of cell membrane to actin cytoskeleton	22ql2.2	Schwann cell tumors, astrocytomas, meningiomas, ependymonas
Familial Adenomatous Polyposis	APC	signaling through adhesion molecules to nucleus	5q21-q22	colon cancer
Tuberous sclerosis 1	TSC1	forms complex with TSC2 protein, inhibits signaling to downstream effectors of mTOR	9q34	seizures, mental retardation, facial angiofibromas
Tuberous sclerosis 2	TSC2	forms complex with TSCl protein, inhibits signaling to downstream effectors of mTOR	16pl3.3	benign growths (hamartomas) in many tissues, astrocytomas, rhabdomyosarcomas
Deleted in Pancreatic Carcinoma 4, Familial juvenile polyposis syndrome	DPC4, also known as SMAD4	regulation of TGF-β/BMP signal transduction	18q21.1	pancreatic carcinoma, colon cancer
Deleted in Colorectal Carcinoma	DCC	transmembrane receptor involved in axonal guidance via netrins	18q21.3	colorectal cancer
Familial Breast Cancer	BRCA1	functions in transcription, DNA binding, transcription coupled DNA repair, homologous recombination, chromosomal stability, ubiquitination of proteins, and centrosome replication	17q21	breast and ovarian cancer

Familial Breast Cancer	BRCA2 (FANCD1)	transcriptional regulation of genes involved in DNA repair and homologous recombination	13ql2.3	breast and ovarian cancer
Cowden syndrome	PTEN	phosphoinositide 3-phosphatase, protein tyrosine phosphatase	10q23.3	gliomas, breast cancer, thyroid cancer, head & neck squamous carcinoma
Peutz-Jeghers Syndrome (PJS)	STK1 1 (serine-threonine kinase 11)	phosphorylates and activates AMP-activated kinase (AMPK), AMPK involved in stress responses, lipid and glucose meatabolism	19pl3.3	hyperpigmentation, multiple hamartomatous polyps, colorectal, breast and ovarian cancers
Hereditary Nonpolyposis Colon Cancer type 1, HNPCCl	MSH2	DNA mismatch repair	2p22-p2 1	colon cancer
Hereditary Nonpolyposis Colon Cancer type 2, HNPCC2	MLH1	DNA mismatch repair	3p21.3	colon cancer
Familial diffuse-type gastric cancer	CDH1	cell-cell adhesion protein	16q22.1	gastric cancer, lobular breast cancer
von Hippel-Lindau Syndrome	VHL	regulation of transcription elongation through activation of a ubiquitin ligase complex	3p26-p25	renal cancers, hemangioblastomas, pheochromocytoma, retinal angioma
Familial Melanoma	CDKN2A	pl6INK4 inhibits cell-cycle kinases CDK4 and CDK6; pl4ARF binds the p53 stabilizing protein MDM2	9p21	melanoma, pancreatic cancer, others

Gorlin Syndrome: Nevoid basal cell carcinoma syndrome (NBCCS)	PTCH (e.g., PTCHl, PTCH2)	transmembrane receptor for sonic hedgehog (shh), involved in early development through repression of action of smoothened	9q22.3	basal cell skin carcinoma
Multiple Endocrine Neoplasia Type 1	MEN1	intrastrand DNA crosslink repair	l lql3	parathyroid and pituitary adenomas, islet cell tumors, carcinoid

[00300] In additional to the above mentioned targets, the the oncology-related polypeptides may include any "death signal" protein that can be recognized by active T cells of immune system. Such suicide signal proteins encoded by the sensor-signal polynucleotides can be selectively expressed in particular tissues or cells (e.g. cancer cells) through engineered microRNA binding sites and/or other regulatory elements as described herein. The group of proteins, when they are expressed on the surface of a caner cell, can prime T cell to induce T cell mediated immune response, thus killing the cancer cell. As a non-limiting example, a group of proteins that are known to present a "death signal", include, CD80, CD86, B7 and MHC II, etc.

Protein Cleavage Signals and Sites

[00301] In one embodiment, the oncology-related polypeptides of the present invention may include at least one protein cleavage signal containing at least one protein cleavage site. The protein cleavage site may be located at the N-terminus, the C-terminus, at any space between the N- and the C- termini such as, but not limited to, half-way between the N- and C-termini, between the N-terminus and the half way point, between the half way point and the C-terminus, and combinations thereof.

[00302] The oncology-related polypeptides of the present invention may include, but is not limited to, a proprotein convertase (or prohormone convertase), thrombin or Factor Xa protein cleavage signal. Proprotein convertases are a family of nine proteinases, comprising seven basic amino acid-specific subtilisin-like serine proteinases related to yeast kexin, known as prohormone convertase 1/3 (PC 1/3), PC2, furin, PC4, PC5/6, paired basic amino-acid cleaving enzyme 4 (PACE4) and PC7, and two other subtilases

that cleave at non-basic residues, called subtilisin kexin isozyme 1 (SKI-1) and proprotein convertase subtilisin kexin 9 (PCSK9). Non-limiting examples of protein cleavage signal amino acid sequences are listing in Table 8. In Table 8, "X" refers to any amino acid, "n" may be 0, 2, 4 or 6 amino acids and "*" refers to the protein cleavage site. In Table 8, SEQ ID NO: 2499 refers to when n=4 and SEQ ID NO: 2500 refers to when n=6.

Table 8. Protein Cleavage Site Sequences

Protein Cleavage Signal	Amino Acid Cleavage Sequence	SEQ ID NO
Proprotein convertase	R-X-X-R*	2497
	R-X-K/R-R*	2498
	K/R-Xn-K/R*	2499 or 2500
Thrombin	L-V-P-R*-G-S	2501
	L-V-P-R*	2502
	A/F/G/I/L/T/V/M-A/F/G/I/L/T/V/W/A-P-R*	2503
Factor Xa	I-E-G-R*	2504
	I-D-G-R*	2505
	A-E-G-R*	2506
	A/F/G/I/L/T/V/M-D/E-G-R*	2507

[00303] In one embodiment, the signal-sensor primary constructs and the mmRNA of the present invention may be engineered such that the primary construct or mmRNA contains at least one encoded protein cleavage signal. The encoded protein cleavage signal may be located before the start codon, after the start codon, before the coding region, within the coding region such as, but not limited to, half way in the coding region, between the start codon and the half way point, between the half way point and the stop codon, after the coding region, before the stop codon, between two stop codons, after the stop codon and combinations thereof.

[00304] In one embodiment, the signal-sensor primary constructs or mmRNA of the present invention may include at least one encoded protein cleavage signal containing at least one protein cleavage site. The encoded protein cleavage signal may include, but is not limited to, a proprotein convertase (or prohormone convertase), thrombin and/or Factor Xa protein cleavage signal. One of skill in the art may use Table 1 above or other known methods to determine the appropriate encoded protein cleavage signal to include

in the signal-sensor primary constructs or mmRNA of the present invention. For example, starting with the signal of Table 8 and considering the codons of Table 1 one can design a signal for the signal-sensor primary construct which can produce a protein signal in the resulting oncology-related polypeptide.

[00305] In one embodiment, the oncology-related polypeptides of the present invention include at least one protein cleavage signal and/or site.

[00306] As a non-limiting example, U.S. Pat. No. 7,374,930 and U.S. Pub. No. 20090227660, herein incorporated by reference in their entireties, use a furin cleavage site to cleave the N-terminal methionine of GLP-1 in the expression product from the Golgi apparatus of the cells. In one embodiment, the polypeptides of the present invention include at least one protein cleavage signal and/or site with the proviso that the polypeptide is not GLP-1.

[00307] In one embodiment, the signal-sensor primary constructs or mmRNA of the present invention includes at least one encoded protein cleavage signal and/or site.

[00308] In one embodiment, the signal-sensor primary constructs or mmRNA of the present invention includes at least one encoded protein cleavage signal and/or site with the proviso that the signal-sensor primary construct or mmRNA does not encode GLP-1 .

[00309] In one embodiment, the signal-sensor primary constructs or mmRNA of the present invention may include more than one coding region. Where multiple coding regions are present in the signal-sensor primary construct or mmRNA of the present invention, the multiple coding regions may be separated by encoded protein cleavage sites. As a non-limiting example, the signal-sensor primary construct or mmRNA may be signed in an ordered pattern. On such pattern follows AXBY form where A and B are coding regions which may be the same or different coding regions and/or may encode the same or different oncology-related polypeptides, and X and Y are encoded protein cleavage signals which may encode the same or different protein cleavage signals. A second such pattern follows the form AXYBZ where A and B are coding regions which may be the same or different coding regions and/or may encode the same or different oncology-related polypeptides, and X, Y and Z are encoded protein cleavage signals which may encode the same or different protein cleavage signals. A third pattern follows the form ABXCY where A, B and C are coding regions which may be the same or

different coding regions and/or may encode the same or different oncology-related polypeptides, and X and Y are encoded protein cleavage signals which may encode the same or different protein cleavage signals.

[00310] In one embodiment, the oncology-related polypeptides, signal-sensor primary constructs and mmRNA can also contain sequences that encode protein cleavage sites so that the polypeptides, signal-sensor primary constructs and mmRNA can be released from a carrier region or a fusion partner by treatment with a specific protease for said protein cleavage site.

microRNA

[00311] microRNAs (or miRNA) are 19-25 nucleotide long noncoding RNAs that bind to the 3'UTR of nucleic acid molecules and down-regulate gene expression either by reducing nucleic acid molecule stability or by inhibiting translation. The modified nucleic acids (mRNA), enhanced modified RNA or ribonucleic acids of the invention may comprise one or more microRNA target sequences, microRNA sequences, or microRNA seeds. Such sequences may correspond to any known microRNA such as those taught in US Publication US2005/0261218 and US Publication US2005/0059005, the contents of which are incorporated herein by reference in their entirety. As a non-limiting embodiment, known microRNAs, their sequences and their binding site sequences in the human genome are listed below in Table 9.

[00312] A microRNA sequence comprises a "seed" region, i.e., a sequence in the region of positions 2-8 of the mature microRNA, which sequence has perfect Watson-Crick complementarity to the miRNA target sequence. A microRNA seed may comprise positions 2-8 or 2-7 of the mature microRNA. In some embodiments, a microRNA seed may comprise 7 nucleotides (e.g., nucleotides 2-8 of the mature microRNA), wherein the seed-complementary site in the corresponding miRNA target is flanked by an adenine (A) opposed to microRNA position 1. In some embodiments, a microRNA seed may comprise 6 nucleotides (e.g., nucleotides 2-7 of the mature microRNA), wherein the seed-complementary site in the corresponding miRNA target is flanked by an adenine (A) opposed to microRNA position 1. See for example, Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Barrel DP; Mol Cell. 2007 Jul 6;27(1):91-105. The bases of the microRNA seed have complete complementarity with the target sequence. By

engineering microRNA target sequences into the 3'UTR of nucleic acids or mRNA of the invention one can target the molecule for degradation or reduced translation, provided the microRNA in question is available. This process will reduce the hazard of off target effects upon nucleic acid molecule delivery. Identification of microRNA, microRNA target regions, and their expression patterns and role in biology have been reported (Bonauer et al., Curr Drug Targets 2010 11:943-949; Anand and Cheresh Curr Opin Hematol 201 1 18:171-176; Contreras and Rao Leukemia 2012 26:404-413 (201 1 Dec 20. doi: 10.1038/leu.201 1.356); Bartel Cell 2009 136:215-233; Landgraf et al, Cell, 2007 129:1401-1414; Gentner andNaldini, Tissue Antigens. 2012 80:393-403 and all references therein; each of which is herein incorporated by reference in its entirety).

[00313] For example, if the signal-sensor polynucleotide is not intended to be delivered to the liver but ends up there, then miR-122, a microRNA abundant in liver, can inhibit the expression of the gene of interest if one or multiple target sites of miR-122 are engineered into the 3'UTR of the signal-sensor polynucleotide. Introduction of one or multiple binding sites for different microRNA can be engineered to further decrease the longevity, stability, and protein translation of a signal-sensor polynucleotide. As used herein, the term "microRNA site" refers to a microRNA target site or a microRNA recognition site, or any nucleotide sequence to which a microRNA binds or associates. It should be understood that "binding" may follow traditional Watson-Crick hybridization rules or may reflect any stable association of the microRNA with the target sequence at or adjacent to the microRNA site.

[00314] Conversely, for the purposes of the signal-sensor polynucleotides of the present invention, microRNA binding sites can be engineered out of (i.e. removed from) sequences in which they naturally occur in order to increase protein expression in specific tissues. For example, miR-122 binding sites may be removed to improve protein expression in the liver.

[00315] In one embodiment, signal-sensor polynucleotides may include at least one miRNA-binding site in the 3'UTR in order to direct cytotoxic or cytoprotective mRNA therapeutics to specific cells such as, but not limited to, normal and/or cancerous cells (e.g., HEP3B or SNU449). As a non-limiting example, a strong apoptotic signal and at least one miR-122a binding site is encoded by the signal-sensor polynucleotide where the

at least one miR-122a binding site is located in the 3'UTR. As another non-limiting example, apoptosis inducing factor short isoform (AIFsh) and at least one miR-122a binding site is encoded by the signal-sensor polynucleotide where the at least one miR-122a binding site is located in the 3'UTR. As yet another non-limiting example, constitutively active (C.A.) caspase 6 and at least one miR-122a binding site is encoded by the signal-sensor polynucleotide where the at least one miR-122a binding site is located in the 3'UTR. As another non-limiting example, HSVl-tk and at least one miR-122a binding site is encoded by the signal-sensor polynucleotide where the at least one miR-122a binding site is located in the 3'UTR.

[00316] In another embodiment, signal-sensor polynucleotides may include three miRNA-binding sites in the 3'UTR in order to direct cytotoxic or cytoprotective mRNA therapeutics to specific cells such as, but not limited to, normal and/or cancerous cells (e.g., HEP3B or SNU449). As a non-limiting example, a strong apoptotic signal and three miR-122a binding sites are encoded by the signal-sensor polynucleotide where the three miR-122a binding sites are located in the 3'UTR. As another non-limiting example, apoptosis inducing factor short isoform (AIFsh) and three miR-122a binding sites are encoded by the signal-sensor polynucleotide where the three miR-122a binding sites are located in the 3'UTR. As yet another non-limiting example, constitutively active (C.A.) caspase 6 and three miR-122a binding sites are encoded by the signal-sensor polynucleotide where the three miR-122a binding sites are located in the 3'UTR. As another non-limiting example, HSVl-tk and and three miR-122a binding sites are encoded by the signal-sensor polynucleotide where the three miR-122a binding sites are located in the 3'UTR.

[00317] Regulation of expression in multiple tissues can be accomplished through introduction or removal or one or several microRNA binding sites. Shown below in Table 10 are microRNAs which are differentially expressed in different tissues and cells, and often associated with different types of dieases (e.g.cancer cells). The decision of removal or insertion of microRNA binding sites, or any combination, is dependent on microRNA expression patterns and their profilings in cancer cells.

[00318] Examples of tissues where microRNA are known to regulate mRNA, and thereby protein expression, include, but are not limited to, liver (miR-122), muscle (miR-

133, miR-206, miR-208), endothelial cells (miR-17-92, miR-126), myeloid cells (miR-142-3p, miR-142-5p, miR-16, miR-21, miR-223, miR-24, miR-27), nervous system (mir-124a, miR-9), pluripotent cells (miR-302, miR-367, miR-290, miR-371, miR-373), pancreatic islet cells (miR-375), adipose tissue (let-7, miR-30c), heart (miR-ld, miR-149), kidney (miR-192, miR-194, miR-204), and lung epithelial cells (let-7, miR-133, miR-126).

[00319] Specifically, microRNAs are known to be differentially expressed in immune cells (also called hematopoietic cells), such as antigen presenting cells (APCs) (e.g. dendritic cells and macrophages), macrophages, monocytes, B lymphocytes, T lymphocytes, granuocytes, natural killer cells, etc. Immune cell specific microRNAs are involved in immunogenicity, autoimmunity, the immune -response to infection, inflammation, as well as unwanted immune response after gene therapy and tissue/organ transplantation. Immune cells specific microRNAs also regulate many aspects of development, proliferation, differentiation and apoptosis of hematopoietic cells (immune cells). For example, miR-142 and miR-146 are exclusively expressed in the immune cells, particularly abundant in myeloid dendritic cells. Introducing the miR-142 binding site into the 3'-UTR of a signal-sensor polypeptide of the present invention can selectively suppress the gene expression in the antigen presenting cells through miR-142 mediated mRNA degradation, limiting antigen presentation in professional APCs (e.g. dendritic cells) and thereby preventing antigen-mediated immune response after gene delivery (see, Annoni A et al, blood, 2009, 114, 5152-5161, the content of which is herein incorporated by reference in its entirety.)

[00320] In one embodiment, microRNAs binding sites that are known to be expressed in immune cells, in particular, the antigen presenting cells, can be engineered into the signal-sensor polynucleotides to suppress the expression of the sensor-signal polynucleotide in APCs through microRNA mediated RNA degradation, subduing the antigen-mediated immune response, while the expression of the sensor-signal polynucleotide is maintained in non-immune cells where the immune cell specific microRNAs are not expressed. For example, to prevent the immunogenic reaction caused by a liver specific protein expression, the miR-122 binding site can be removed and the miR-142 (and/or mirR-146) binding sites can be engineered into the 3-UTR of the signal

-sensor polynucleotide (e.g., see the constructs described in Example 38 and the experiment outlined in Examples 39 and 40).

[00321] To further drive the selective degradation and suppression of mRNA in APCs and macrophage, the signal-sensor polynucleotide may include another negative regulatory element in the 3-UTR, either alone or in combination with mir-142 and/or mir-146 binding sites. As a non-limiting example, one regulatory element is the Constitutive Decay Elements (CDEs).

[00322] Immune cells specific microRNAs include, but are not limited to, hsa-let-7a-2-3p, hsa-let-7a-3p, hsa-7a-5p, hsa-let-7c, hsa-let-7e-3p, hsa-let-7e-5p, hsa-let-7g-3p, hsa-let-7g-5p, hsa-let-7i-3p, hsa-let-7i-5p, miR-10a-3p, miR-10a-5p, miR-1 184, hsa-let-7f-l —3p, hsa-let-7f-2~5p, hsa-let-7f-5p, miR-125b-l-3p, miR-125b-2-3p, miR-125b-5p, miR-1279, miR-130a-3p, miR-130a-5p, miR-132-3p, miR-132-5p, miR-142-3p, miR-142-5p, miR-143-3p, miR-143-5p, miR-146a-3p, miR-146a-5p, miR-146b-3p, miR-146b-5p, miR-147a, miR-147b, miR-148a-5p, miR-148a-3p, miR-150-3p, miR-150-5p, miR-151b, miR-155-3p, miR-155-5p, miR-15a-3p, miR-15a-5p, miR-15b-5p, miR-15b-3p, miR-16-l-3p, miR-16-2-3p, miR-16-5p, miR-17-5p, miR-181a-3p, miR-181a-5p, miR-181a-2-3p, miR-182-3p, miR-182-5p, miR-197-3p, miR-197-5p, miR-21-5p, miR-21-3p, miR-214-3p, miR-214-5p, miR-223-3p, miR-223-5p, miR-221-3p, miR-221-5p, miR-23b-3p, miR-23b-5p, miR-24-l-5p,miR-24-2-5p, miR-24-3p, miR-26a-l-3p, miR-26a-2-3p, miR-26a-5p, miR-26b-3p, miR-26b-5p, miR-27a-3p, miR-27a-5p, miR-27b-3p,miR-27b-5p, miR-28-3p, miR-28-5p, miR-2909, miR-29a-3p, miR-29a-5p, miR-29b-l-5p, miR-29b-2-5p, miR-29c-3p, miR-29c-5p,, miR-30e-3p, miR-30e-5p, miR-331-5p, miR-339-3p, miR-339-5p, miR-345-3p, miR-345-5p, miR-346, miR-34a-3p, miR-34a-5p, , miR-363-3p, miR-363-5p, miR-372, miR-377-3p, miR-377-5p, miR-493-3p, miR-493-5p, miR-542, miR-548b-5p, miR548c-5p, miR-548i, miR-548j, miR-548n, miR-574-3p, miR-598, miR-718, miR-935, miR-99a-3p, miR-99a-5p, miR-99b-3p and miR-99b-5p. Shown below in Table 11 are microRNAs that are enriched in specific types of immune cells. Furthermore, novel miroRNAs are discovered in the immune cells in the art through micro-array hybridization and microtome analysis (Jima DD et al, Blood, 2010, 116:el 18-el27; Vaz C et al, BMC Genomics, 2010, 11,288, the content of each of which is incorporated herein by reference in its entirety).

[00323] MicroRNAs that are known to be expressed in the liver include, but are not limited to, miR-107, miR-122-3p, miR-122-5p, miR-1228-3p, miR-1228-5p, miR-1249, miR-129-5p, miR-1303, miR-151a-3p, miR-151a-5p, miR-152, miR-194-3p, miR-194-5p, miR-199a-3p, miR-199a-5p, miR-199b-3p, miR-199b-5p, miR-296-5p, miR-557, miR-581, miR-939-3p, miR-939-5p. microRNA binding sites from any liver specific microRNA can be introduced to or removed from the signal-sensor polynucleotides to regulate the expression of the signal-sensor polynucleotides in the liver. Liver specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites in order to prevent an immune reaction against protein expression in the liver.

[00324] MicroRNAs that are known to be expressed in the lung include, but are not limited to, let-7a-2-3p, let-7a-3p, let-7a-5p, miR-126-3p, miR-126-5p, miR-127-3p, miR-127-5p, miR-130a-3p, miR-130a-5p, miR-130b-3p, miR-130b-5p, miR-133a, miR-133b, miR-134, miR-18a-3p, miR-18a-5p, miR-18b-3p, miR-18b-5p, miR-24-l-5p, miR-24-2-5p, miR-24-3p, miR-296-3p, miR-296-5p, miR-32-3p, miR-337-3p, miR-337-5p, miR-381-3p, miR-381-5p. MicroRNA binding sites from any lung specific microRNA can be introduced to or removed from the signal-sensor polynucleotide to regulate the expression of the signal-sensor polynucleotide in the lung. Lung specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites in order to prevent an immune reaction against protein expression in the lung.

[00325] MicroRNAs that are known to be expressed in the heart include, but are not limited to, miR-1, miR-133a, miR-133b, miR-149-3p, miR-149-5p, miR-186-3p, miR-186-5p, miR-208a, miR-208b, miR-210, miR-296-3p, miR-320, miR-451a, miR-451b, miR-499a-3p, miR-499a-5p, miR-499b-3p, miR-499b-5p, miR-744-3p, miR-744-5p, miR-92b-3p and miR-92b-5p. microRNA binding sites from any heart specific microRNA can be introduced to or removed from the signal-sensor polynucleotides to regulate the expression of the signal-sensor polynucleotides in the heart. Heart specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites in order to prevent an immune reaction against protein expression in the heart.

[00326] MicroRNAs that are known to be expressed in the nervous system include, but are not limited to, miR-124-5p, miR-125a-3p, miR-125a-5p, miR-125b-l-3p, miR-125b-2-3p, miR-125b-5p,miR-1271-3p, miR-1271-5p, miR-128, miR-132-5p, miR-135a-3p, miR-135a-5p, miR-135b-3p, miR-135b-5p, miR-137, miR-139-5p, miR-139-3p, miR-149-3p, miR-149-5p, miR-153, miR-181c-3p, miR-181c-5p, miR-183-3p, miR-183-5p, miR-190a, miR-190b, miR-212-3p, miR-212-5p, miR-219-l-3p, miR-219-2-3p, miR-23a-3p, miR-23a-5p,miR-30a-5p, miR-30b-3p, miR-30b-5p, miR-30c-l-3p, miR-30c-2-3p, miR-30c-5p, miR-30d-3p, miR-30d-5p, miR-329, miR-342-3p, miR-3665, miR-3666, miR-380-3p, miR-380-5p, miR-383, miR-410, miR-425-3p, miR-425-5p, miR-454-3p, miR-454-5p, miR-483, miR-510, miR-516a-3p, miR-548b-5p, miR-548c-5p, miR-571, miR-7-l-3p, miR-7-2-3p, miR-7-5p, miR-802, miR-922, miR-9-3p and miR-9-5p. microRNAs enriched in the nervous system further include those specifically expressed in neurons, including, but not limited to, miR-132-3p, miR-132-3p, miR-148b-3p, miR-148b-5p, miR-151a-3p, miR-151a-5p, miR-212-3p, miR-212-5p, miR-320b, miR-320e, miR-323a-3p, miR-323a-5p, miR-324-5p, miR-325, miR-326, miR-328, miR-922 and those specifically expressed in glial cells, including, but not limited to, miR-1250, miR-219-l-3p, miR-219-2-3p, miR-219-5p, miR-23a-3p, miR-23a-5p, miR-3065-3p, miR-3065-5p, miR-30e-3p, miR-30e-5p, miR-32-5p, miR-338-5p, miR-657. microRNA binding sites from any CNS specific microRNA can be introduced to or removed from the signal-sensor polynucleotides to regulate the expression of the signal-sensor polynucleotide in the nervous system. Nervous system specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites in order to prevent an immune reaction against protein expression in the nervous system.

[00327] MicroRNAs that are known to be expressed in the pancreas include, but are not limited to, miR-105-3p, miR-105-5p, miR-184, miR-195-3p, miR-195-5p, miR-196a-3p, miR-196a-5p, miR-214-3p, miR-214-5p, miR-216a-3p, miR-216a-5p, miR-30a-3p, miR-33a-3p, miR-33a-5p, miR-375, miR-7-l-3p, miR-7-2-3p, miR-493-3p, miR-493-5p and miR-944. MicroRNA binding sites from any pancreas specific microRNA can be introduced to or removed from the signal-sensor polynucleotide to regulate the expression of the signal-sensor polynucleotide in the pancreas. Pancreas specific

microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites in order to prevent immune reaction against protein expression in the pancreas.

[00328] MicroRNAs that are known to be expressed in the kidney further include, but are not limited to, miR-122-3p, miR-145-5p, miR-17-5p, miR-192-3p, miR-192-5p, miR-194-3p, miR-194-5p, miR-20a-3p, miR-20a-5p, miR-204-3p, miR-204-5p, miR-210, miR-216a-3p, miR-216a-5p, miR-296-3p, miR-30a-3p, miR-30a-5p, miR-30b-3p, miR-30b-5p, miR-30c-l-3p, miR-30c-2-3p, miR30c-5p, miR-324-3p, miR-335-3p, miR-335-5p, miR-363-3p, miR-363-5p and miR-562. MicroRNA binding sites from any kidney specific microRNA can be introduced to or removed from the signal-sensor polynucleotide to regulate the expression of the signal-sensor polynucleotide in the kidney. Kidney specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites in order to prevent immune reaction against protein expression in the kidney.

[00329] MicroRNAs that are known to be expressed in the muscle further include, but are not limited to, let-7g-3p, let-7g-5p, miR-1, miR-1286, miR-133a, miR-133b, miR-140-3p, miR-143-3p, miR-143-5p, miR-145-3p, miR-145-5p, miR-188-3p, miR-188-5p, miR-206, miR-208a, miR-208b, miR-25-3p and miR-25-5p. MicroRNA binding sites from any muscle specific microRNA can be introduced to or removed from the signal-sensor polynucleotide to regulate the expression of the signal-sensor polynucleotide in the muscle. Muscle specific microRNAs binding sites can be engineered alone or further in combination with immune cells (e.g. APCs) microRNA binding sites in order to prevent an immune reaction against protein expression in the muscle.

[00330] MicroRNAs are differentially expressed in different types of cells, such as endothelial cells, epithelial cells and adipocytes. For example, microRNAs that are expressed in endothelial cells include, but are not limited to, let-7b-3p, let-7b-5p, miR-100-3p, miR-100-5p, miR-101-3p, miR-101-5p, miR-126-3p, miR-126-5p, miR-1236-3p, miR-1236-5p, miR-130a-3p, miR-130a-5p, miR-17-5p, miR-17-3p, miR-18a-3p, miR-18a-5p, , miR-19a-3p, miR-19a-5p, miR-19b-l-5p, miR-19b-2-5p, miR-19b-3p, miR-20a-3p, miR-20a-5p, miR-217, miR-210, miR-21-3p, miR-21-5p, miR-221-3p, miR-221-5p, miR-222-3p, miR-222-5p, miR-23a-3p, miR-23a-5p, miR-296-5p, miR-361-3p, miR-

361-5p, miR-421, miR-424-3p, miR-424-5p, miR-513a-5p, miR-92a-l-5p, miR-92a-2-5p, miR-92a-3p, miR-92b-3p and miR-92b-5p. Many novel microRNAs were discovered in endothelial cells from deep-sequencing analysis (Voellenkle C e tal, RNA, 2012, 18, 472-484, herein incorporated by reference in its entirety). MicroRNA binding sites from any endothelial cell specific microRNA can be introduced to or removed from the signal-sensor polynucleotide in order to modulate the expression of the signal-sensor polynucleotide in the endothelial cells in various conditions.

[00331] For further example, microRNAs that are expressed in epithelial cells include, but are not limited to, let-7b-3p, let-7b-5p, miR- 1246, miR-200a-3p, miR-200a-5p, miR-200b-3p, miR-200b-5p, miR-200c-3p, miR-200c-5p, miR-338-3p, miR-429, miR-451a, miR-451b, miR-494, miR-802 and miR-34a, miR-34b-5p , miR-34c-5p, miR-449a, miR-449b-3p, miR-449b-5p specific in respiratory ciliated epithelial cells; let-7 family, miR-133a, miR-133b, miR-126 specific in lung epithelial cells; miR-382-3p, miR-382-5p specific in renal epithelial cells and miR-762 specific in corneal epithelial cells. MicroRNA binding sites from any epithelial cell specific microRNA can be introduced to or removed from the signal-sensor polynucleotide in order to modulate the expression of the signal-sensor polynucleotide in the epithelial cells in various conditions.

[00332] In addition, a large group of microRNAs are enriched in embryonic stem cells, controlling stem cell self-renewal as well as the development and/or differentiation of various cell lineages, such as neural cells, cardiac, hematopoietic cells, skin cells, osteogenic cells and muscle cells (Kuppusamy KT et al, Curr. Mol Med, 2013, 13(5), 757-764; Vidigal JA and Ventura A, Semin Cancer Biol. 2012, 22(5-6), 428-436; Goff LA et al, PLoS One, 2009, 4:e7192; Morin RD et al, Genome Res,2008,18, 610-621; Yoo JK et al, Stem Cells Dev. 2012, 21(1 1), 2049-2057, each of which is herein incorporated by reference in its entirety). MicroRNAs abundant in embryonic stem cells include, but are not limited to, let-7a-2-3p, let-a-3p, let-7a-5p, let7d-3p, let-7d-5p, miR-103a-2-3p, miR-103a-5p, miR-106b-3p, miR-106b-5p, miR-1246, miR-1275, miR-138-l-3p, miR-138-2-3p, miR-138-5p, miR-154-3p, miR-154-5p, miR-200c-3p, miR-200c-5p, miR-290, miR-301a-3p, miR-301a-5p, miR-302a-3p, miR-302a-5p, miR-302b-3p, miR-302b-5p, miR-302c-3p, miR-302c-5p, miR-302d-3p, miR-302d-5p, miR-302e, miR-367-3p, miR-367-5p, miR-369-3p, miR-369-5p, miR-370, miR-371, miR-373, miR-380-

5p, miR-423-3p, miR-423-5p, miR-486-5p, miR-520c-3p, miR-548e, miR-548f, miR-548g-3p, miR-548g-5p, miR-548i, miR-548k, miR-5481, miR-548m, miR-548n, miR-548o-3p, miR-548o-5p, miR-548p, miR-664a-3p, miR-664a-5p, miR-664b-3p, miR-664b-5p, miR-766-3p, miR-766-5p, miR-885-3p, miR-885-5p,miR-93-3p, miR-93-5p, miR-941,miR-96-3p, miR-96-5p, miR-99b-3p and miR-99b-5p. Many predicted novel microRNAs are discovered by deep sequencing in human embryonic stem cells (Morin RD et al, Genome Res,2008,18, 610-621; Goff LA et al, PLoS One, 2009, 4:e7192; Bar M et al, Stem cells, 2008, 26, 2496-2505, the content of each of which is incorporated herein by references in its entirety).

[00333] In one embodiment, the binding sites of embryonic stem cell specific microRNAs can be included in or removed from the 3-UTR of the signal-sensor polynucleotide to modulate the development and/or differentiation of embryonic stem cells, to inhibit the senescence of stem cells in a degenerative condition (e.g. degenerative diseases), or to stimulate the senescence and apoptosis of stem cells in a disease condition (e.g. cancer stem cell).

[00334] Many microRNA expression studies have been conducted, and are described in the art, to profile the differential expression of microRNAs in various cancer cells /tissues and other diseases. Some microRNAs are abnormally over-expressed in certain cancer cells and others are under-expressed. For example, microRNAs are differentially expressed in cancer cells (WO2008/154098, US2013/0059015, US2013/0042333, WO201 1/157294); cancer stem cells (US2012/0053224); pancreatic cancers and diseases (US2009/0131348, US201 1/0171646, US20 10/0286232, US8389210); asthma and inflammation (US84 15096); prostate cancer (US20 13/0053264); hepatocellular carcinoma (WO2012/151212, US2012/0329672, WO2008/054828, US8252538); lung cancer cells (WO201 1/076143, WO20 13/033640, WO2009/070653, US2010/0323357); cutaneous T cell lymphoma (WO2013/01 1378); colorectal cancer cells (WO20 11/0281756, WO201 1/076142); cancer positive lympho nodes (WO2009/1 00430, US2009/0263803); nasopharyngeal carcinoma (EP21 12235); chronic obstructive pulmonary disease (US20 12/0264626, US2013/0053263); thyroid cancer (WO20 13/066678); ovarian cancer cells (US2012/0309645, WO201 1/095623); breast cancer cells (WO2008/1 54098, WO2007/081740, US20 12/02 14699), leukemia and

lymphoma (WO2008/073915, US2009/0092974, US20 12/03 16081, US2012/0283310, WO2010/018563, the content of each of which is incorporated herein by reference in their entirety).

[00335] Specifically, microRNA sites that are over-expressed in certain cancer and/or tumor cells can be removed from the 3-UTR of the signal-sensor polynucleotide encoding the oncology-related polypeptide, restoring the expression suppressed by the over-expressed microRNAs in cancer cells, thus ameliorating the corresponsive biological function, for instance, transcription stimulation and/or repression, cell cycle arrest, apoptosis and cell death. Normal cells and tissues, wherein microRNA expression is not up-regulated, will remain unaffected.

[00336] MicroRNA can also regulate complex biological processes such as angiogenesis (miR-132) (Anand and Cheresh Curr Opin Hematol 201 1 18:171-176). In the signal-sensor polynucleotides of the invention, binding sites for microRNAs that are involved in such processes may be removed or introduced, in order to tailor the expression of the signal-sensor polynucleotides expression to biologically relevant cell types or to the context of relevant biological processes. In this context, the signal-sensor polynucleotideare defined as auxotrophic signal-sensor polynucleotides.

[00337] Table 9 is a non-exhaustive listing of miRs and miR binding sites (miR BS) and their sequences which may be used with the present invention.

Table 9. Mirs and mir binding sites

microRNA	mir SEQ ID	BS SEQ ID	microRNA	mir SEQ ID	BS SEQ ID
hsa-let-7a-2-3p	2508	3529	hsa-miR-4471	4550	5571
hsa-let-7a-3p	2509	3530	hsa-miR-4472	4551	5572
hsa-let-7a-5p	2510	3531	hsa-miR-4473	4552	5573
hsa-let-7b-3p	2511	3532	hsa-miR-4474-3p	4553	5574
hsa-let-7b-5p	2512	3533	hsa-miR-4474-5p	4554	5575
hsa-let-7c	2513	3534	hsa-miR-4475	4555	5576
hsa-let-7d-3p	2514	3535	hsa-miR-4476	4556	5577
hsa-let-7d-5p	2515	3536	hsa-miR-4477a	4557	5578
hsa-let-7e-3p	2516	3537	hsa-miR-4477b	4558	5579
hsa-let-7e-5p	2517	3538	hsa-miR-4478	4559	5580
hsa-let-7f-1-3p	2518	3539	hsa-miR-4479	4560	5581
hsa-let-7f-2-3p	2519	3540	hsa-miR-448	4561	5582
hsa-let-7f-5p	2520	3541	hsa-miR-4480	4562	5583
hsa-let-7g-3p	2521	3542	hsa-miR-4481	4563	5584
hsa-let-7g-5p	2522	3543	hsa-miR-4482-3p	4564	5585

hsa-let-7i-3p	2523	3544	hsa-miR-4482-5p	4565	5586
hsa-let-7i-5p	2524	3545	hsa-miR-4483	4566	5587
hsa-miR- 1	2525	3546	hsa-miR-4484	4567	5588
hsa-miR- 100-3p	2526	3547	hsa-miR-4485	4568	5589
hsa-miR- 100-5p	2527	3548	hsa-miR-4486	4569	5590
hsa-miR- 10 l-3p	2528	3549	hsa-miR-4487	4570	5591
hsa-miR- 10 l-5p	2529	3550	hsa-miR-4488	4571	5592
hsa-miR- 103a-2-5p	2530	3551	hsa-miR-4489	4572	5593
hsa-miR- 103a-3p	2531	3552	hsa-miR-4490	4573	5594
hsa-miR- 103b	2532	3553	hsa-miR-4491	4574	5595
hsa-miR- 105-3p	2533	3554	hsa-miR-4492	4575	5596
hsa-miR- 105-5p	2534	3555	hsa-miR-4493	4576	5597
hsa-miR- 106a-3p	2535	3556	hsa-miR-4494	4577	5598
hsa-miR- 106a-5p	2536	3557	hsa-miR-4495	4578	5599
hsa-miR- 106b-3p	2537	3558	hsa-miR-4496	4579	5600
hsa-miR- 106b-5p	2538	3559	hsa-miR-4497	4580	5601
hsa-miR- 107	2539	3560	hsa-miR-4498	4581	5602
hsa-miR- 10a-3p	2540	3561	hsa-miR-4499	4582	5603
hsa-miR- 10a-5p	2541	3562	hsa-miR-449a	4583	5604
hsa-miR- 10b-3p	2542	3563	hsa-miR-449b-3p	4584	5605
hsa-miR- 10b-5p	2543	3564	hsa-miR-449b-5p	4585	5606
hsa-miR- 1178-3p	2544	3565	hsa-miR-449c-3p	4586	5607
hsa-miR- 1178-5p	2545	3566	hsa-miR-449c-5p	4587	5608
hsa-miR- 1179	2546	3567	hsa-miR-4500	4588	5609
hsa-miR- 1180	2547	3568	hsa-miR-4501	4589	5610
hsa-miR-1181	2548	3569	hsa-miR-4502	4590	561 1
hsa-miR- 1182	2549	3570	hsa-miR-4503	4591	5612
hsa-miR- 1183	2550	3571	hsa-miR-4504	4592	5613
hsa-miR- 1184	2551	3572	hsa-miR-4505	4593	5614
hsa-miR- 1185- l-3p	2552	3573	hsa-miR-4506	4594	5615
hsa-miR- 1185-2-3p	2553	3574	hsa-miR-4507	4595	5616
hsa-miR- 1185-5p	2554	3575	hsa-miR-4508	4596	5617
hsa-miR- 1193	2555	3576	hsa-miR-4509	4597	5618
hsa-miR- 1197	2556	3577	hsa-miR-450a-3p	4598	5619
hsa-miR- 1200	2557	3578	hsa-miR-450a-5p	4599	5620
hsa-miR- 1202	2558	3579	hsa-miR-450b-3p	4600	5621
hsa-miR- 1203	2559	3580	hsa-miR-450b-5p	4601	5622
hsa-miR- 1204	2560	3581	hsa-miR-4510	4602	5623
hsa-miR- 1205	2561	3582	hsa-miR-451 1	4603	5624
hsa-miR- 1206	2562	3583	hsa-miR-4512	4604	5625
hsa-miR- 1207-3p	2563	3584	hsa-miR-4513	4605	5626
hsa-miR- 1207-5p	2564	3585	hsa-miR-4514	4606	5627
hsa-miR- 1208	2565	3586	hsa-miR-4515	4607	5628
hsa-miR- 122-3p	2566	3587	hsa-miR-4516	4608	5629
hsa-miR- 1224-3p	2567	3588	hsa-miR-4517	4609	5630
hsa-miR- 1224-5p	2568	3589	hsa-miR-4518	4610	5631
hsa-miR- 1225-3p	2569	3590	hsa-miR-4519	461 1	5632
hsa-miR- 1225-5p	2570	3591	hsa-miR-45 1a	4612	5633
hsa-miR- 122-5p	2571	3592	hsa-miR-451b	4613	5634

hsa-miR- 1226-3p	2572	3593	hsa-miR-4520a-3p	4614	5635
hsa-miR- 1226-5p	2573	3594	hsa-miR-4520a-5p	4615	5636
hsa-miR- 1227-3p	2574	3595	hsa-miR-4520b-3p	4616	5637
hsa-miR- 1227-5p	2575	3596	hsa-miR-4520b-5p	4617	5638
hsa-miR- 1228-3p	2576	3597	hsa-miR-4521	4618	5639
hsa-miR- 1228-5p	2577	3598	hsa-miR-4522	4619	5640
hsa-miR- 1229-3p	2578	3599	hsa-miR-4523	4620	5641
hsa-miR- 1229-5p	2579	3600	hsa-miR-452-3p	4621	5642
hsa-miR- 1231	2580	3601	hsa-miR-4524a-3p	4622	5643
hsa-miR- 1233 - 1-5p	2581	3602	hsa-miR-4524a-5p	4623	5644
hsa-miR- 1233-3p	2582	3603	hsa-miR-4524b-3p	4624	5645
hsa-miR- 1234-3p	2583	3604	hsa-miR-4524b-5p	4625	5646
hsa-miR- 1234-5p	2584	3605	hsa-miR-4525	4626	5647
hsa-miR- 1236-3p	2585	3606	hsa-miR-452-5p	4627	5648
hsa-miR- 1236-5p	2586	3607	hsa-miR-4526	4628	5649
hsa-miR- 1237-3p	2587	3608	hsa-miR-4527	4629	5650
hsa-miR- 1237-5p	2588	3609	hsa-miR-4528	4630	5651
hsa-miR- 1238-3p	2589	3610	hsa-miR-4529-3p	4631	5652
hsa-miR- 1238-5p	2590	361 1	hsa-miR-4529-5p	4632	5653
hsa-miR- 1243	2591	3612	hsa-miR-4530	4633	5654
hsa-miR- 124-3p	2592	3613	hsa-miR-453 1	4634	5655
hsa-miR- 1244	2593	3614	hsa-miR-4532	4635	5656
hsa-miR- 1245a	2594	3615	hsa-miR-4533	4636	5657
hsa-miR- 1245b-3p	2595	3616	hsa-miR-4534	4637	5658
hsa-miR- 1245b-5p	2596	3617	hsa-miR-4535	4638	5659
hsa-miR- 124-5p	2597	3618	hsa-miR-4536-3p	4639	5660
hsa-miR- 1246	2598	3619	hsa-miR-4536-5p	4640	5661
hsa-miR- 1247-3p	2599	3620	hsa-miR-4537	4641	5662
hsa-miR- 1247-5p	2600	3621	hsa-miR-453 8	4642	5663
hsa-miR- 1248	2601	3622	hsa-miR-4539	4643	5664
hsa-miR- 1249	2602	3623	hsa-miR-4540	4644	5665
hsa-miR- 1250	2603	3624	hsa-miR-454-3p	4645	5666
hsa-miR- 1251	2604	3625	hsa-miR-454-5p	4646	5667
hsa-miR- 1252	2605	3626	hsa-miR-455-3p	4647	5668
hsa-miR- 1253	2606	3627	hsa-miR-455-5p	4648	5669
hsa-miR- 1254	2607	3628	hsa-miR-4632-3p	4649	5670
hsa-miR- 1255a	2608	3629	hsa-miR-4632-5p	4650	5671
hsa-miR- 1255b-2-3p	2609	3630	hsa-miR-4633-3p	4651	5672
hsa-miR- 1255b-5p	2610	3631	hsa-miR-4633-5p	4652	5673
hsa-miR- 1256	261 1	3632	hsa-miR-4634	4653	5674
hsa-miR- 1257	2612	3633	hsa-miR-4635	4654	5675
hsa-miR- 1258	2613	3634	hsa-miR-4636	4655	5676
hsa-miR- 125a-3p	2614	3635	hsa-miR-4637	4656	5677
hsa-miR- 125a-5p	2615	3636	hsa-miR-4638-3p	4657	5678
hsa-miR- 125b- 1-3p	2616	3637	hsa-miR-4638-5p	4658	5679
hsa-miR- 125b-2-3p	2617	3638	hsa-miR-4639-3p	4659	5680
hsa-miR- 125b-5p	2618	3639	hsa-miR-4639-5p	4660	5681
hsa-miR- 1260a	2619	3640	hsa-miR-4640-3p	4661	5682
hsa-miR- 1260b	2620	3641	hsa-miR-4640-5p	4662	5683

hsa-miR- 1261	2621	3642	hsa-miR-4641	4663	5684
hsa-miR- 1262	2622	3643	hsa-miR-4642	4664	5685
hsa-miR- 1263	2623	3644	hsa-miR-4643	4665	5686
hsa-miR- 126-3p	2624	3645	hsa-miR-4644	4666	5687
hsa-miR- 1264	2625	3646	hsa-miR-4645-3p	4667	5688
hsa-miR- 1265	2626	3647	hsa-miR-4645-5p	4668	5689
hsa-miR- 126-5p	2627	3648	hsa-miR-4646-3p	4669	5690
hsa-miR- 1266	2628	3649	hsa-miR-4646-5p	4670	5691
hsa-miR- 1267	2629	3650	hsa-miR-4647	4671	5692
hsa-miR- 1268a	2630	3651	hsa-miR-4648	4672	5693
hsa-miR- 1268b	2631	3652	hsa-miR-4649-3p	4673	5694
hsa-miR- 1269a	2632	3653	hsa-miR-4649-5p	4674	5695
hsa-miR- 1269b	2633	3654	hsa-miR-4650-3p	4675	5696
hsa-miR- 1270	2634	3655	hsa-miR-4650-5p	4676	5697
hsa-miR- 127 l-3p	2635	3656	hsa-miR-465 1	4677	5698
hsa-miR- 127 l-5p	2636	3657	hsa-miR-4652-3p	4678	5699
hsa-miR- 1272	2637	3658	hsa-miR-4652-5p	4679	5700
hsa-miR- 1273 a	2638	3659	hsa-miR-4653-3p	4680	5701
hsa-miR- 1273c	2639	3660	hsa-miR-4653-5p	4681	5702
hsa-miR- 1273 d	2640	3661	hsa-miR-4654	4682	5703
hsa-miR- 1273 e	2641	3662	hsa-miR-4655-3p	4683	5704
hsa-miR- 1273 f	2642	3663	hsa-miR-4655-5p	4684	5705
hsa-miR- 1273g-3p	2643	3664	hsa-miR-4656	4685	5706
hsa-miR- 1273g-5p	2644	3665	hsa-miR-4657	4686	5707
hsa-miR- 127-3p	2645	3666	hsa-miR-465 8	4687	5708
hsa-miR- 1275	2646	3667	hsa-miR-4659a-3p	4688	5709
hsa-miR- 127-5p	2647	3668	hsa-miR-4659a-5p	4689	5710
hsa-miR- 1276	2648	3669	hsa-miR-4659b-3p	4690	571 1
hsa-miR- 1277-3p	2649	3670	hsa-miR-4659b-5p	4691	5712
hsa-miR- 1277-5p	2650	3671	hsa-miR-466	4692	5713
hsa-miR- 1278	2651	3672	hsa-miR-4660	4693	5714
hsa-miR- 1279	2652	3673	hsa-miR-466 l-3p	4694	5715
hsa-miR- 128	2653	3674	hsa-miR-466 l-5p	4695	5716
hsa-miR- 1281	2654	3675	hsa-miR-4662a-3p	4696	5717
hsa-miR- 1282	2655	3676	hsa-miR-4662a-5p	4697	5718
hsa-miR- 1283	2656	3677	hsa-miR-4662b	4698	5719
hsa-miR- 1284	2657	3678	hsa-miR-4663	4699	5720
hsa-miR- 1285-3p	2658	3679	hsa-miR-4664-3p	4700	5721
hsa-miR- 1285-5p	2659	3680	hsa-miR-4664-5p	4701	5722
hsa-miR- 1286	2660	3681	hsa-miR-4665-3p	4702	5723
hsa-miR- 1287	2661	3682	hsa-miR-4665-5p	4703	5724
hsa-miR- 1288	2662	3683	hsa-miR-4666a-3p	4704	5725
hsa-miR- 1289	2663	3684	hsa-miR-4666a-5p	4705	5726
hsa-miR- 1290	2664	3685	hsa-miR-4666b	4706	5727
hsa-miR- 1291	2665	3686	hsa-miR-4667-3p	4707	5728
hsa-miR- 129- l-3p	2666	3687	hsa-miR-4667-5p	4708	5729
hsa-miR- 1292-3p	2667	3688	hsa-miR-4668-3p	4709	5730
hsa-miR- 129-2-3p	2668	3689	hsa-miR-4668-5p	4710	5731
hsa-miR- 1292-5p	2669	3690	hsa-miR-4669	471 1	5732

hsa-miR-1293	2670	3691	hsa-miR-4670-3p	4712	5733
hsa-miR-1294	2671	3692	hsa-miR-4670-5p	4713	5734
hsa-miR- 1295a	2672	3693	hsa-miR-4671-3p	4714	5735
hsa-miR-1295b-3p	2673	3694	hsa-miR-4671-5p	4715	5736
hsa-miR- 1295b-5p	2674	3695	hsa-miR-4672	4716	5737
hsa-miR- 129-5p	2675	3696	hsa-miR-4673	4717	5738
hsa-miR- 1296	2676	3697	hsa-miR-4674	4718	5739
hsa-miR- 1297	2677	3698	hsa-miR-4675	4719	5740
hsa-miR- 1298	2678	3699	hsa-miR-4676-3p	4720	5741
hsa-miR- 1299	2679	3700	hsa-miR-4676-5p	4721	5742
hsa-miR- 1301	2680	3701	hsa-miR-4677-3p	4722	5743
hsa-miR- 1302	2681	3702	hsa-miR-4677-5p	4723	5744
hsa-miR- 1303	2682	3703	hsa-miR-4678	4724	5745
hsa-miR- 1304-3p	2683	3704	hsa-miR-4679	4725	5746
hsa-miR- 1304-5p	2684	3705	hsa-miR-4680-3p	4726	5747
hsa-miR- 1305	2685	3706	hsa-miR-4680-5p	4727	5748
hsa-miR- 1306-3p	2686	3707	hsa-miR-4681	4728	5749
hsa-miR- 1306-5p	2687	3708	hsa-miR-4682	4729	5750
hsa-miR- 1307-3p	2688	3709	hsa-miR-4683	4730	5751
hsa-miR- 1307-5p	2689	3710	hsa-miR-4684-3p	4731	5752
hsa-miR- 130a-3p	2690	371 1	hsa-miR-4684-5p	4732	5753
hsa-miR- 130a-5p	2691	3712	hsa-miR-4685-3p	4733	5754
hsa-miR- 130b-3p	2692	3713	hsa-miR-4685-5p	4734	5755
hsa-miR- 130b-5p	2693	3714	hsa-miR-4686	4735	5756
hsa-miR- 1321	2694	3715	hsa-miR-4687-3p	4736	5757
hsa-miR- 1322	2695	3716	hsa-miR-4687-5p	4737	5758
hsa-miR- 1323	2696	3717	hsa-miR-4688	4738	5759
hsa-miR- 132-3p	2697	3718	hsa-miR-4689	4739	5760
hsa-miR- 1324	2698	3719	hsa-miR-4690-3p	4740	5761
hsa-miR- 132-5p	2699	3720	hsa-miR-4690-5p	4741	5762
hsa-miR- 133a	2700	3721	hsa-miR-4691-3p	4742	5763
hsa-miR- 133b	2701	3722	hsa-miR-4691-5p	4743	5764
hsa-miR- 134	2702	3723	hsa-miR-4692	4744	5765
hsa-miR- 1343	2703	3724	hsa-miR-4693-3p	4745	5766
hsa-miR- 135a-3p	2704	3725	hsa-miR-4693-5p	4746	5767
hsa-miR- 135a-5p	2705	3726	hsa-miR-4694-3p	4747	5768
hsa-miR- 135b-3p	2706	3727	hsa-miR-4694-5p	4748	5769
hsa-miR- 135b-5p	2707	3728	hsa-miR-4695-3p	4749	5770
hsa-miR- 136-3p	2708	3729	hsa-miR-4695-5p	4750	5771
hsa-miR- 136-5p	2709	3730	hsa-miR-4696	4751	5772
hsa-miR- 137	2710	3731	hsa-miR-4697-3p	4752	5773
hsa-miR- 13 8- l-3p	271 1	3732	hsa-miR-4697-5p	4753	5774
hsa-miR- 138-2-3p	2712	3733	hsa-miR-4698	4754	5775
hsa-miR- 138-5p	2713	3734	hsa-miR-4699-3p	4755	5776
hsa-miR- 139-3p	2714	3735	hsa-miR-4699-5p	4756	5777
hsa-miR- 139-5p	2715	3736	hsa-miR-4700-3p	4757	5778
hsa-miR- 140-3p	2716	3737	hsa-miR-4700-5p	4758	5779
hsa-miR- 140-5p	2717	3738	hsa-miR-4701-3p	4759	5780
hsa-miR- 14 l-3p	2718	3739	hsa-miR-4701-5p	4760	5781

hsa-miR-141-5p	2719	3740	hsa-miR-4703-3p	4761	5782
hsa-miR-142-3p	2720	3741	hsa-miR-4703-5p	4762	5783
hsa-miR-142-5p	2721	3742	hsa-miR-4704-3p	4763	5784
hsa-miR-143-3p	2722	3743	hsa-miR-4704-5p	4764	5785
hsa-miR-143-5p	2723	3744	hsa-miR-4705	4765	5786
hsa-miR-144-3p	2724	3745	hsa-miR-4706	4766	5787
hsa-miR-144-5p	2725	3746	hsa-miR-4707-3p	4767	5788
hsa-miR-145-3p	2726	3747	hsa-miR-4707-5p	4768	5789
hsa-miR-145-5p	2727	3748	hsa-miR-4708-3p	4769	5790
hsa-miR-1468	2728	3749	hsa-miR-4708-5p	4770	5791
hsa-miR-1469	2729	3750	hsa-miR-4709-3p	4771	5792
hsa-miR-146a-3p	2730	3751	hsa-miR-4709-5p	4772	5793
hsa-miR-146a-5p	2731	3752	hsa-miR-4710	4773	5794
hsa-miR-146b-3p	2732	3753	hsa-miR-471 1-3p	4774	5795
hsa-miR-146b-5p	2733	3754	hsa-miR-471 1-5p	4775	5796
hsa-miR-1470	2734	3755	hsa-miR-4712-3p	4776	5797
hsa-miR-1471	2735	3756	hsa-miR-4712-5p	4777	5798
hsa-miR-147a	2736	3757	hsa-miR-4713-3p	4778	5799
hsa-miR-147b	2737	3758	hsa-miR-4713-5p	4779	5800
hsa-miR-148a-3p	2738	3759	hsa-miR-4714-3p	4780	5801
hsa-miR-148a-5p	2739	3760	hsa-miR-4714-5p	4781	5802
hsa-miR-148b-3p	2740	3761	hsa-miR-4715-3p	4782	5803
hsa-miR-148b-5p	2741	3762	hsa-miR-4715-5p	4783	5804
hsa-miR-149-3p	2742	3763	hsa-miR-4716-3p	4784	5805
hsa-miR-149-5p	2743	3764	hsa-miR-4716-5p	4785	5806
hsa-miR-150-3p	2744	3765	hsa-miR-4717-3p	4786	5807
hsa-miR-150-5p	2745	3766	hsa-miR-4717-5p	4787	5808
hsa-miR- 151 a-3p	2746	3767	hsa-miR-4718	4788	5809
hsa-miR- 151 a-5p	2747	3768	hsa-miR-4719	4789	5810
hsa-miR- 15 lb	2748	3769	hsa-miR-4720-3p	4790	581 1
hsa-miR- 152	2749	3770	hsa-miR-4720-5p	4791	5812
hsa-miR- 153	2750	3771	hsa-miR-4721	4792	5813
hsa-miR- 1537	2751	3772	hsa-miR-4722-3p	4793	5814
hsa-miR- 1538	2752	3773	hsa-miR-4722-5p	4794	5815
hsa-miR- 1539	2753	3774	hsa-miR-4723-3p	4795	5816
hsa-miR- 154-3p	2754	3775	hsa-miR-4723-5p	4796	5817
hsa-miR- 154-5p	2755	3776	hsa-miR-4724-3p	4797	5818
hsa-miR- 155-3p	2756	3777	hsa-miR-4724-5p	4798	5819
hsa-miR- 155-5p	2757	3778	hsa-miR-4725-3p	4799	5820
hsa-miR- 1587	2758	3779	hsa-miR-4725-5p	4800	5821
hsa-miR- 15a-3p	2759	3780	hsa-miR-4726-3p	4801	5822
hsa-miR- 15a-5p	2760	3781	hsa-miR-4726-5p	4802	5823
hsa-miR- 15b-3p	2761	3782	hsa-miR-4727-3p	4803	5824
hsa-miR- 15b-5p	2762	3783	hsa-miR-4727-5p	4804	5825
hsa-miR- 16- l-3p	2763	3784	hsa-miR-4728-3p	4805	5826
hsa-miR- 16-2-3p	2764	3785	hsa-miR-4728-5p	4806	5827
hsa-miR- 16-5p	2765	3786	hsa-miR-4729	4807	5828
hsa-miR- 17-3p	2766	3787	hsa-miR-4730	4808	5829
hsa-miR- 17-5p	2767	3788	hsa-miR-4731-3p	4809	5830

hsa-miR-181a-2-3p	2768	3789	hsa-miR-4731-5p	4810	5831
hsa-miR-181a-3p	2769	3790	hsa-miR-4732-3p	4811	5832
hsa-miR-181a-5p	2770	3791	hsa-miR-4732-5p	4812	5833
hsa-miR-181b-3p	2771	3792	hsa-miR-4733-3p	4813	5834
hsa-miR-181b-5p	2772	3793	hsa-miR-4733-5p	4814	5835
hsa-miR-181c-3p	2773	3794	hsa-miR-4734	4815	5836
hsa-miR-181c-5p	2774	3795	hsa-miR-4735-3p	4816	5837
hsa-miR-181d	2775	3796	hsa-miR-4735-5p	4817	5838
hsa-miR-182-3p	2776	3797	hsa-miR-4736	4818	5839
hsa-miR-1825	2777	3798	hsa-miR-4737	4819	5840
hsa-miR-182-5p	2778	3799	hsa-miR-4738-3p	4820	5841
hsa-miR-1827	2779	3800	hsa-miR-4738-5p	4821	5842
hsa-miR-183-3p	2780	3801	hsa-miR-4739	4822	5843
hsa-miR-183-5p	2781	3802	hsa-miR-4740-3p	4823	5844
hsa-miR-184	2782	3803	hsa-miR-4740-5p	4824	5845
hsa-miR-185-3p	2783	3804	hsa-miR-4741	4825	5846
hsa-miR-185-5p	2784	3805	hsa-miR-4742-3p	4826	5847
hsa-miR-186-3p	2785	3806	hsa-miR-4742-5p	4827	5848
hsa-miR-186-5p	2786	3807	hsa-miR-4743-3p	4828	5849
hsa-miR-187-3p	2787	3808	hsa-miR-4743-5p	4829	5850
hsa-miR-187-5p	2788	3809	hsa-miR-4744	4830	5851
hsa-miR-188-3p	2789	3810	hsa-miR-4745-3p	4831	5852
hsa-miR-188-5p	2790	3811	hsa-miR-4745-5p	4832	5853
hsa-miR-18a-3p	2791	3812	hsa-miR-4746-3p	4833	5854
hsa-miR-18a-5p	2792	3813	hsa-miR-4746-5p	4834	5855
hsa-miR-18b-3p	2793	3814	hsa-miR-4747-3p	4835	5856
hsa-miR-18b-5p	2794	3815	hsa-miR-4747-5p	4836	5857
hsa-miR-1908	2795	3816	hsa-miR-4748	4837	5858
hsa-miR-1909-3p	2796	3817	hsa-miR-4749-3p	4838	5859
hsa-miR-1909-5p	2797	3818	hsa-miR-4749-5p	4839	5860
hsa-miR-190a	2798	3819	hsa-miR-4750-3p	4840	5861
hsa-miR-190b	2799	3820	hsa-miR-4750-5p	4841	5862
hsa-miR-1910	2800	3821	hsa-miR-4751	4842	5863
hsa-miR-1911-3p	2801	3822	hsa-miR-4752	4843	5864
hsa-miR-1911-5p	2802	3823	hsa-miR-4753-3p	4844	5865
hsa-miR-1912	2803	3824	hsa-miR-4753-5p	4845	5866
hsa-miR-1913	2804	3825	hsa-miR-4754	4846	5867
hsa-miR-191-3p	2805	3826	hsa-miR-4755-3p	4847	5868
hsa-miR-1914-3p	2806	3827	hsa-miR-4755-5p	4848	5869
hsa-miR-1914-5p	2807	3828	hsa-miR-4756-3p	4849	5870
hsa-miR-1915-3p	2808	3829	hsa-miR-4756-5p	4850	5871
hsa-miR-1915-5p	2809	3830	hsa-miR-4757-3p	4851	5872
hsa-miR-191-5p	2810	3831	hsa-miR-4757-5p	4852	5873
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hsa-miR-192-5p	2812	3833	hsa-miR-4758-5p	4854	5875
hsa-miR-193a-3p	2813	3834	hsa-miR-4759	4855	5876
hsa-miR-193a-5p	2814	3835	hsa-miR-4760-3p	4856	5877
hsa-miR-193b-3p	2815	3836	hsa-miR-4760-5p	4857	5878
hsa-miR-193b-5p	2816	3837	hsa-miR-4761-3p	4858	5879

hsa-miR-194-3p	2817	3838	hsa-miR-4761-5p	4859	5880
hsa-miR-194-5p	2818	3839	hsa-miR-4762-3p	4860	5881
hsa-miR-195-3p	2819	3840	hsa-miR-4762-5p	4861	5882
hsa-miR-195-5p	2820	3841	hsa-miR-4763-3p	4862	5883
hsa-miR-196a-3p	2821	3842	hsa-miR-4763-5p	4863	5884
hsa-miR-196a-5p	2822	3843	hsa-miR-4764-3p	4864	5885
hsa-miR-196b-3p	2823	3844	hsa-miR-4764-5p	4865	5886
hsa-miR-196b-5p	2824	3845	hsa-miR-4765	4866	5887
hsa-miR-1972	2825	3846	hsa-miR-4766-3p	4867	5888
hsa-miR-1973	2826	3847	hsa-miR-4766-5p	4868	5889
hsa-miR-197-3p	2827	3848	hsa-miR-4767	4869	5890
hsa-miR-197-5p	2828	3849	hsa-miR-4768-3p	4870	5891
hsa-miR-1976	2829	3850	hsa-miR-4768-5p	4871	5892
hsa-miR-198	2830	3851	hsa-miR-4769-3p	4872	5893
hsa-miR-199a-3p	2831	3852	hsa-miR-4769-5p	4873	5894
hsa-miR-199a-5p	2832	3853	hsa-miR-4770	4874	5895
hsa-miR-199b-3p	2833	3854	hsa-miR-4771	4875	5896
hsa-miR-199b-5p	2834	3855	hsa-miR-4772-3p	4876	5897
hsa-miR- 19a-3p	2835	3856	hsa-miR-4772-5p	4877	5898
hsa-miR- 19a-5p	2836	3857	hsa-miR-4773	4878	5899
hsa-miR- 19b- l-5p	2837	3858	hsa-miR-4774-3p	4879	5900
hsa-miR- 19b-2-5p	2838	3859	hsa-miR-4774-5p	4880	5901
hsa-miR- 19b-3p	2839	3860	hsa-miR-4775	4881	5902
hsa-miR-200a-3p	2840	3861	hsa-miR-4776-3p	4882	5903
hsa-miR-200a-5p	2841	3862	hsa-miR-4776-5p	4883	5904
hsa-miR-200b-3p	2842	3863	hsa-miR-4777-3p	4884	5905
hsa-miR-200b-5p	2843	3864	hsa-miR-4777-5p	4885	5906
hsa-miR-200c-3p	2844	3865	hsa-miR-4778-3p	4886	5907
hsa-miR-200c-5p	2845	3866	hsa-miR-4778-5p	4887	5908
hsa-miR-202-3p	2846	3867	hsa-miR-4779	4888	5909
hsa-miR-202-5p	2847	3868	hsa-miR-4780	4889	5910
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hsa-miR-203b-5p	2850	3871	hsa-miR-4782-3p	4892	5913
hsa-miR-204-3p	2851	3872	hsa-miR-4782-5p	4893	5914
hsa-miR-204-5p	2852	3873	hsa-miR-4783-3p	4894	5915
hsa-miR-2052	2853	3874	hsa-miR-4783-5p	4895	5916
hsa-miR-2053	2854	3875	hsa-miR-4784	4896	5917
hsa-miR-205-3p	2855	3876	hsa-miR-4785	4897	5918
hsa-miR-2054	2856	3877	hsa-miR-4786-3p	4898	5919
hsa-miR-205-5p	2857	3878	hsa-miR-4786-5p	4899	5920
hsa-miR-206	2858	3879	hsa-miR-4787-3p	4900	5921
hsa-miR-208a	2859	3880	hsa-miR-4787-5p	4901	5922
hsa-miR-208b	2860	3881	hsa-miR-4788	4902	5923
hsa-miR-20a-3p	2861	3882	hsa-miR-4789-3p	4903	5924
hsa-miR-20a-5p	2862	3883	hsa-miR-4789-5p	4904	5925
hsa-miR-20b-3p	2863	3884	hsa-miR-4790-3p	4905	5926
hsa-miR-20b-5p	2864	3885	hsa-miR-4790-5p	4906	5927
hsa-miR-210	2865	3886	hsa-miR-4791	4907	5928

hsa-miR-21 10	2866	3887	hsa-miR-4792	4908	5929
hsa-miR-21 13	2867	3888	hsa-miR-4793-3p	4909	5930
hsa-miR-21 l-3p	2868	3889	hsa-miR-4793-5p	4910	5931
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hsa-miR-21 14-5p	2870	3891	hsa-miR-4795-3p	4912	5933
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hsa-miR-21 15-5p	2872	3893	hsa-miR-4796-3p	4914	5935
hsa-miR-21 l-5p	2873	3894	hsa-miR-4796-5p	4915	5936
hsa-miR-21 16-3p	2874	3895	hsa-miR-4797-3p	4916	5937
hsa-miR-21 16-5p	2875	3896	hsa-miR-4797-5p	4917	5938
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hsa-miR-21 2-3p	2877	3898	hsa-miR-4798-5p	4919	5940
hsa-miR-21 2-5p	2878	3899	hsa-miR-4799-3p	4920	5941
hsa-miR-21 -3p	2879	3900	hsa-miR-4799-5p	4921	5942
hsa-miR-21 4-3p	2880	3901	hsa-miR-4800-3p	4922	5943
hsa-miR-21 4-5p	2881	3902	hsa-miR-4800-5p	4923	5944
hsa-miR-21 5	2882	3903	hsa-miR-4801	4924	5945
hsa-miR-21 -5p	2883	3904	hsa-miR-4802-3p	4925	5946
hsa-miR-21 6a-3p	2884	3905	hsa-miR-4802-5p	4926	5947
hsa-miR-2 16a-5p	2885	3906	hsa-miR-4803	4927	5948
hsa-miR-21 6b	2886	3907	hsa-miR-4804-3p	4928	5949
hsa-miR-2 17	2887	3908	hsa-miR-4804-5p	4929	5950
hsa-miR-21 8- l-3p	2888	3909	hsa-miR-483-3p	4930	5951
hsa-miR-21 8-2-3p	2889	3910	hsa-miR-483-5p	4931	5952
hsa-miR-21 8-5p	2890	391 1	hsa-miR-484	4932	5953
hsa-miR-21 9- l-3p	2891	3912	hsa-miR-485-3p	4933	5954
hsa-miR-2 19-2-3p	2892	3913	hsa-miR-485-5p	4934	5955
hsa-miR-2 19-5p	2893	3914	hsa-miR-486-3p	4935	5956
hsa-miR-221-3p	2894	3915	hsa-miR-486-5p	4936	5957
hsa-miR-221-5p	2895	3916	hsa-miR-487a	4937	5958
hsa-miR-222-3p	2896	3917	hsa-miR-487b	4938	5959
hsa-miR-222-5p	2897	3918	hsa-miR-488-3p	4939	5960
hsa-miR-223-3p	2898	3919	hsa-miR-488-5p	4940	5961
hsa-miR-223-5p	2899	3920	hsa-miR-489	4941	5962
hsa-miR-22-3p	2900	3921	hsa-miR-490-3p	4942	5963
hsa-miR-224-3p	2901	3922	hsa-miR-490-5p	4943	5964
hsa-miR-224-5p	2902	3923	hsa-miR-491-3p	4944	5965
hsa-miR-22-5p	2903	3924	hsa-miR-491-5p	4945	5966
hsa-miR-2276	2904	3925	hsa-miR-492	4946	5967
hsa-miR-2277-3p	2905	3926	hsa-miR-493-3p	4947	5968
hsa-miR-2277-5p	2906	3927	hsa-miR-493-5p	4948	5969
hsa-miR-2278	2907	3928	hsa-miR-494	4949	5970
hsa-miR-2355-3p	2908	3929	hsa-miR-495-3p	4950	5971
hsa-miR-2355-5p	2909	3930	hsa-miR-495-5p	4951	5972
hsa-miR-2392	2910	3931	hsa-miR-496	4952	5973
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hsa-miR-23b-3p	2913	3934	hsa-miR-498	4955	5976
hsa-miR-23b-5p	2914	3935	hsa-miR-4999-3p	4956	5977

hsa-miR-23c	2915	3936	hsa-miR-4999-5p	4957	5978
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hsa-miR-24-2-5p	2917	3938	hsa-miR-499a-5p	4959	5980
hsa-miR-24-3p	2918	3939	hsa-miR-499b-3p	4960	5981
hsa-miR-2467-3p	2919	3940	hsa-miR-499b-5p	4961	5982
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hsa-miR-25-3p	2921	3942	hsa-miR-5000-5p	4963	5984
hsa-miR-25-5p	2922	3943	hsa-miR-5001-3p	4964	5985
hsa-miR-2681-3p	2923	3944	hsa-miR-5001-5p	4965	5986
hsa-miR-2681-5p	2924	3945	hsa-miR-5002-3p	4966	5987
hsa-miR-2682-3p	2925	3946	hsa-miR-5002-5p	4967	5988
hsa-miR-2682-5p	2926	3947	hsa-miR-5003-3p	4968	5989
hsa-miR-26a- 1-3p	2927	3948	hsa-miR-5003-5p	4969	5990
hsa-miR-26a-2-3p	2928	3949	hsa-miR-5004-3p	4970	5991
hsa-miR-26a-5p	2929	3950	hsa-miR-5004-5p	4971	5992
hsa-miR-26b-3p	2930	3951	hsa-miR-5006-3p	4972	5993
hsa-miR-26b-5p	2931	3952	hsa-miR-5006-5p	4973	5994
hsa-miR-27a-3p	2932	3953	hsa-miR-5007-3p	4974	5995
hsa-miR-27a-5p	2933	3954	hsa-miR-5007-5p	4975	5996
hsa-miR-27b-3p	2934	3955	hsa-miR-5008-3p	4976	5997
hsa-miR-27b-5p	2935	3956	hsa-miR-5008-5p	4977	5998
hsa-miR-28-3p	2936	3957	hsa-miR-5009-3p	4978	5999
hsa-miR-28-5p	2937	3958	hsa-miR-5009-5p	4979	6000
hsa-miR-2861	2938	3959	hsa-miR-500a-3p	4980	6001
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hsa-miR-296-3p	2940	3961	hsa-miR-500b	4982	6003
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hsa-miR-3621	3186	4207	hsa-miR-5590-3p	5228	6249
hsa-miR-3622a-3p	3187	4208	hsa-miR-5590-5p	5229	6250
hsa-miR-3622a-5p	3188	4209	hsa-miR-559 l-3p	5230	6251
hsa-miR-3622b-3p	3189	4210	hsa-miR-559 l-5p	5231	6252
hsa-miR-3622b-5p	3190	421 1	hsa-miR-561-3p	5232	6253
hsa-miR-362-3p	3191	4212	hsa-miR-561-5p	5233	6254
hsa-miR-362-5p	3192	4213	hsa-miR-562	5234	6255
hsa-miR-363-3p	3193	4214	hsa-miR-563	5235	6256
hsa-miR-363-5p	3194	4215	hsa-miR-564	5236	6257
hsa-miR-3646	3195	4216	hsa-miR-566	5237	6258
hsa-miR-3648	3196	4217	hsa-miR-567	5238	6259
hsa-miR-3649	3197	4218	hsa-miR-568	5239	6260
hsa-miR-3650	3198	4219	hsa-miR-5680	5240	6261
hsa-miR-3651	3199	4220	hsa-miR-568 l a	5241	6262
hsa-miR-3652	3200	4221	hsa-miR-568 l b	5242	6263
hsa-miR-3653	3201	4222	hsa-miR-5682	5243	6264
hsa-miR-3654	3202	4223	hsa-miR-5683	5244	6265
hsa-miR-3655	3203	4224	hsa-miR-5684	5245	6266
hsa-miR-3656	3204	4225	hsa-miR-5685	5246	6267
hsa-miR-3657	3205	4226	hsa-miR-5686	5247	6268
hsa-miR-3658	3206	4227	hsa-miR-5687	5248	6269
hsa-miR-3659	3207	4228	hsa-miR-5688	5249	6270
hsa-miR-365a-3p	3208	4229	hsa-miR-5689	5250	6271

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hsa-miR-365b-5p	321 1	4232	hsa-miR-569 1	5253	6274
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hsa-miR-3662	3214	4235	hsa-miR-5692c	5256	6277
hsa-miR-3663-3p	3215	4236	hsa-miR-5693	5257	6278
hsa-miR-3663-5p	3216	4237	hsa-miR-5694	5258	6279
hsa-miR-3664-3p	3217	4238	hsa-miR-5695	5259	6280
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hsa-miR-3666	3220	4241	hsa-miR-5698	5262	6283
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hsa-miR-3672	3227	4248	hsa-miR-5704	5269	6290
hsa-miR-3673	3228	4249	hsa-miR-5705	5270	6291
hsa-miR-367-3p	3229	4250	hsa-miR-570-5p	5271	6292
hsa-miR-3674	3230	4251	hsa-miR-5706	5272	6293
hsa-miR-3675-3p	3231	4252	hsa-miR-5707	5273	6294
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hsa-miR-3678-3p	3238	4259	hsa-miR-574-5p	5280	6301
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hsa-miR-3682-3p	3246	4267	hsa-miR-580	5288	6309
hsa-miR-3682-5p	3247	4268	hsa-miR-581	5289	6310
hsa-miR-3683	3248	4269	hsa-miR-582-3p	5290	631 1
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hsa-miR-3686	3251	4272	hsa-miR-584-3p	5293	6314
hsa-miR-3687	3252	4273	hsa-miR-584-5p	5294	6315
hsa-miR-3688-3p	3253	4274	hsa-miR-585	5295	6316
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hsa-miR-3689a-5p	3256	4277	hsa-miR-588	5298	6319
hsa-miR-3689b-3p	3257	4278	hsa-miR-589-3p	5299	6320

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hsa-miR-3692-3p	3266	4287	hsa-miR-596	5308	6329
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hsa-miR-371b-5p	3276	4297	hsa-miR-606	5318	6339
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hsa-miR-374c-5p	3285	4306	hsa-miR-6075	5327	6348
hsa-miR-375	3286	4307	hsa-miR-6076	5328	6349
hsa-miR-376a-2-5p	3287	4308	hsa-miR-6077	5329	6350
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hsa-miR-377-5p	3295	4316	hsa-miR-6084	5337	6358
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hsa-miR-378a-5p	3297	4318	hsa-miR-6086	5339	6360
hsa-miR-378b	3298	4319	hsa-miR-6087	5340	6361
hsa-miR-378c	3299	4320	hsa-miR-6088	5341	6362
hsa-miR-378d	3300	4321	hsa-miR-6089	5342	6363
hsa-miR-378e	3301	4322	hsa-miR-609	5343	6364
hsa-miR-378f	3302	4323	hsa-miR-6090	5344	6365
hsa-miR-378g	3303	4324	hsa-miR-610	5345	6366
hsa-miR-378h	3304	4325	hsa-miR-6 11	5346	6367
hsa-miR-378i	3305	4326	hsa-miR-612	5347	6368
hsa-miR-378j	3306	4327	hsa-miR-6 124	5348	6369

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hsa-miR-382-5p	3314	4335	hsa-miR-6 131	5356	6377
hsa-miR-383	3315	4336	hsa-miR-6 132	5357	6378
hsa-miR-384	3316	4337	hsa-miR-6 133	5358	6379
hsa-miR-3907	3317	4338	hsa-miR-6 134	5359	6380
hsa-miR-3908	3318	4339	hsa-miR-6 14	5360	6381
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hsa-miR-3913-5p	3324	4345	hsa-miR-6 17	5366	6387
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hsa-miR-3920	3331	4352	hsa-miR-624-3p	5373	6394
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hsa-miR-3922-3p	3333	4354	hsa-miR-625-3p	5375	6396
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hsa-miR-3923	3335	4356	hsa-miR-626	5377	6398
hsa-miR-3924	3336	4357	hsa-miR-627	5378	6399
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hsa-miR-3941	3353	4374	hsa-miR-642a-3p	5395	6416
hsa-miR-3942-3p	3354	4375	hsa-miR-642a-5p	5396	6417
hsa-miR-3942-5p	3355	4376	hsa-miR-642b-3p	5397	6418

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hsa-miR-3944-5p	3358	4379	hsa-miR-644a	5400	6421
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hsa-miR-3960	3360	4381	hsa-miR-646	5402	6423
hsa-miR-3972	3361	4382	hsa-miR-647	5403	6424
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hsa-miR-3974	3363	4384	hsa-miR-649	5405	6426
hsa-miR-3975	3364	4385	hsa-miR-6499-3p	5406	6427
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hsa-miR-3978	3367	4388	hsa-miR-6500-3p	5409	6430
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hsa-miR-4262	3393	4414	hsa-miR-65 1lb-5p	5435	6456
hsa-miR-4263	3394	4415	hsa-miR-65 12-3p	5436	6457
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hsa-miR-429	3421	4442	hsa-miR-664b-3p	5463	6484
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hsa-miR-4294	3426	4447	hsa-miR-67 l-3p	5468	6489
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hsa-miR-4303	3435	4456	hsa-miR-67 19-3p	5477	6498
hsa-miR-4304	3436	4457	hsa-miR-6720-3p	5478	6499
hsa-miR-4305	3437	4458	hsa-miR-6721-5p	5479	6500
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hsa-miR-4309	3441	4462	hsa-miR-6724-5p	5483	6504
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hsa-miR-43 l-3p	3446	4467	hsa-miR-708-3p	5488	6509
hsa-miR-43 14	3447	4468	hsa-miR-708-5p	5489	6510
hsa-miR-43 15	3448	4469	hsa-miR-7 11	5490	651 1
hsa-miR-43 l-5p	3449	4470	hsa-miR-7-l-3p	5491	6512
hsa-miR-43 16	3450	4471	hsa-miR-7 18	5492	6513
hsa-miR-43 17	3451	4472	hsa-miR-7-2-3p	5493	6514
hsa-miR-43 18	3452	4473	hsa-miR-744-3p	5494	6515
hsa-miR-43 19	3453	4474	hsa-miR-744-5p	5495	6516

hsa-miR-4320	3454	4475	hsa-miR-758-3p	5496	6517
hsa-miR-4321	3455	4476	hsa-miR-758-5p	5497	6518
hsa-miR-4322	3456	4477	hsa-miR-759	5498	6519
hsa-miR-4323	3457	4478	hsa-miR-7-5p	5499	6520
hsa-miR-432-3p	3458	4479	hsa-miR-760	5500	6521
hsa-miR-4324	3459	4480	hsa-miR-76 1	5501	6522
hsa-miR-4325	3460	4481	hsa-miR-762	5502	6523
hsa-miR-432-5p	3461	4482	hsa-miR-764	5503	6524
hsa-miR-4326	3462	4483	hsa-miR-765	5504	6525
hsa-miR-4327	3463	4484	hsa-miR-766-3p	5505	6526
hsa-miR-4328	3464	4485	hsa-miR-766-5p	5506	6527
hsa-miR-4329	3465	4486	hsa-miR-767-3p	5507	6528
hsa-miR-433	3466	4487	hsa-miR-767-5p	5508	6529
hsa-miR-4330	3467	4488	hsa-miR-769-3p	5509	6530
hsa-miR-4417	3468	4489	hsa-miR-769-5p	5510	6531
hsa-miR-4418	3469	4490	hsa-miR-770-5p	551 1	6532
hsa-miR-4419a	3470	4491	hsa-miR-802	5512	6533
hsa-miR-4419b	3471	4492	hsa-miR-873-3p	5513	6534
hsa-miR-4420	3472	4493	hsa-miR-873-5p	5514	6535
hsa-miR-4421	3473	4494	hsa-miR-874	5515	6536
hsa-miR-4422	3474	4495	hsa-miR-875-3p	5516	6537
hsa-miR-4423-3p	3475	4496	hsa-miR-875-5p	5517	6538
hsa-miR-4423-5p	3476	4497	hsa-miR-876-3p	5518	6539
hsa-miR-4424	3477	4498	hsa-miR-876-5p	5519	6540
hsa-miR-4425	3478	4499	hsa-miR-877-3p	5520	6541
hsa-miR-4426	3479	4500	hsa-miR-877-5p	5521	6542
hsa-miR-4427	3480	4501	hsa-miR-885-3p	5522	6543
hsa-miR-4428	3481	4502	hsa-miR-885-5p	5523	6544
hsa-miR-4429	3482	4503	hsa-miR-887	5524	6545
hsa-miR-4430	3483	4504	hsa-miR-888-3p	5525	6546
hsa-miR-443 1	3484	4505	hsa-miR-888-5p	5526	6547
hsa-miR-4432	3485	4506	hsa-miR-889	5527	6548
hsa-miR-4433-3p	3486	4507	hsa-miR-890	5528	6549
hsa-miR-4433-5p	3487	4508	hsa-miR-891a	5529	6550
hsa-miR-4434	3488	4509	hsa-miR-891b	5530	6551
hsa-miR-443 5	3489	4510	hsa-miR-892a	5531	6552
hsa-miR-443 6a	3490	451 1	hsa-miR-892b	5532	6553
hsa-miR-4436b-3p	3491	4512	hsa-miR-892c-3p	5533	6554
hsa-miR-4436b-5p	3492	4513	hsa-miR-892c-5p	5534	6555
hsa-miR-443 7	3493	4514	hsa-miR-920	5535	6556
hsa-miR-443 8	3494	4515	hsa-miR-921	5536	6557
hsa-miR-443 9	3495	4516	hsa-miR-922	5537	6558
hsa-miR-4440	3496	4517	hsa-miR-924	5538	6559
hsa-miR-4441	3497	4518	hsa-miR-92a- 1-5p	5539	6560
hsa-miR-4442	3498	4519	hsa-miR-92a-2-5p	5540	6561
hsa-miR-4443	3499	4520	hsa-miR-92a-3p	5541	6562
hsa-miR-4444	3500	4521	hsa-miR-92b-3p	5542	6563
hsa-miR-4445-3p	3501	4522	hsa-miR-92b-5p	5543	6564
hsa-miR-4445-5p	3502	4523	hsa-miR-933	5544	6565

hsa-miR-4446-3p	3503	4524	hsa-miR-93-3p	5545	6566
hsa-miR-4446-5p	3504	4525	hsa-miR-934	5546	6567
hsa-miR-4447	3505	4526	hsa-miR-935	5547	6568
hsa-miR-4448	3506	4527	hsa-miR-93-5p	5548	6569
hsa-miR-4449	3507	4528	hsa-miR-936	5549	6570
hsa-miR-4450	3508	4529	hsa-miR-937-3p	5550	6571
hsa-miR-445 1	3509	4530	hsa-miR-937-5p	5551	6572
hsa-miR-4452	3510	4531	hsa-miR-938	5552	6573
hsa-miR-4453	351 1	4532	hsa-miR-939-3p	5553	6574
hsa-miR-4454	3512	4533	hsa-miR-939-5p	5554	6575
hsa-miR-4455	3513	4534	hsa-miR-9-3p	5555	6576
hsa-miR-4456	3514	4535	hsa-miR-940	5556	6577
hsa-miR-4457	3515	4536	hsa-miR-941	5557	6578
hsa-miR-445 8	3516	4537	hsa-miR-942	5558	6579
hsa-miR-4459	3517	4538	hsa-miR-943	5559	6580
hsa-miR-4460	3518	4539	hsa-miR-944	5560	6581
hsa-miR-4461	3519	4540	hsa-miR-95	5561	6582
hsa-miR-4462	3520	4541	hsa-miR-9-5p	5562	6583
hsa-miR-4463	3521	4542	hsa-miR-96-3p	5563	6584
hsa-miR-4464	3522	4543	hsa-miR-96-5p	5564	6585
hsa-miR-4465	3523	4544	hsa-miR-98-3p	5565	6586
hsa-miR-4466	3524	4545	hsa-miR-98-5p	5566	6587
hsa-miR-4467	3525	4546	hsa-miR-99a-3p	5567	6588
hsa-miR-4468	3526	4547	hsa-miR-99a-5p	5568	6589
hsa-miR-4469	3527	4548	hsa-miR-99b-3p	5569	6590
hsa-miR-4470	3528	4549	hsa-miR-99b-5p	5570	6591

[00338] As shown in Table 10, microRNAs are differentially expressed in different tissues and cells, and often associated with different types of dieases (e.g. cancer cells). The decision of removal or insertion of microRNA binding sites, or any combination, is dependent on microRNA expression patterns and their profilings in cancer cells. In Table 10, "HCC" represents hepatocellular carcinoma, "ALL" stands for acute lymphoblastsic leukemia, "RCC" stands for renal cell carcinoma, "CLL" stands for chrominc lymphocytic leukemia and "MALT" stands for mucosa-associated lymphoid tissue.

Table 10. mirs, tissues/cell expression and diseases

microRNA	mir SEQ ID	BS SEQ ID	Tissues/cells	Associated Disease	Biological Function
hsa-let-7a-2-3p	2508	3529	Embryonic stem cells, lung, myeloid cells	inflammatory, various cancers (lung, cervical, breast, pancreatic,	tumor suppressor

				etc)	
hsa-let-7a-3p	2509	3530	Embryonic stem cells, lung	inflammatory, various cancers (lung, cervical, breast, pancreatic, etc)	tumor suppressor
hsa-let-7a-5p	2510	3531	Embryonic stem cells, lung	inflammatory, various cancers (lung, cervical, breast, pancreatic, etc)	tumor suppressor
hsa-let-7b-3p	2511	3532	epithelial cells, endothelial cells (vascular)	lung cancer, colorectal cancer, cervical cancer, inflammation and immune response after infection	tumor angiogenesis
hsa-let-7b-5p	2512	3533	epithelial cells, endothelial cells (vascular)	cervical cancer, inflammation and immune response after infection	tumor angiogenesis
hsa-let-7c	2513	3534	dendritic cells	various cacners (cervical, pancreatic, lung , esopphageal, etc)	tumor suppressor, apoptosis
hsa-let-7d-3p	2514	3535	embryonic stem cells	associated with various cancer cells	tumor suppressor
hsa-let-7d-5p	2515	3536	embryonic stem cells	associated with various cancer cells	tumor suppressor
hsa-let-7e-3p	2516	3537	immune cells	various cancer cells, autoimmunity, endotoxin tolerance	tumor suppressor
hsa-let-7e-5p	2517	3538	immune cells	various cancer cells	tumor suppressor
hsa-let-7f-1-3p	2518	3539	immune cells (T cells)	various cancer cells	tumor suppressor
hsa-let-7f-2-3p	2519	3540	immune cells (T cells)	various cancer cells	tumor suppressor
hsa-let-7f-5p	2520	3541	immune cells (T cells)	Various cancer cells	tumor suppressor
hsa-let-7g-3p	2521	3542	hematopoietic cells, adipose, smooth muscle cells	various cancer cells (lung, breast, etc)	tumor suppressor
hsa-let-7g-5p	2522	3543	hematopoietic cells, adipose, smooth muscle cells	various cancer cells (lung, breast, etc)	tumor suppressor
hsa-let-7i-3p	2523	3544	immune cells	chronic lymphocyte leukimia	tumor suppressor
hsa-let-7i-5p	2524	3545	immune cells	chronic	tumor

				lymphocyte leukimia	suppressor
hsa-miR-1	2525	3546	muscle, heart		angiogenesis, cell proliferation(myogenesis)
hsa-miR-100-3p	2526	3547	hematopoietic cells, endothelial cells	gastric cancer, pancreatic cancer	tumor angiogenesis
hsa-miR-100-5p	2527	3548	hematopoietic cells, endothelial cells	gastric cancer, pancreatic cancer	tumor angiogenesis
hsa-miR-101-3p	2528	3549	endothelial cells	various cancers (breast,non-small cell lung, colon, gastric, pancreatic, bladder, etc); lupus erythematosus	angiogenesis
hsa-miR-101-5p	2529	3550	endothelial cells	various cancers (breast,non-small cell lung, colon, gastric, pancreatic, bladder, etc); lupus erythematosus	angiogenesis
hsa-miR-103a-2-5p	2530	3551	embryonic stem cells, many tissues/cells	various cancers (endometrial, neuroblastoma, colorectal, breast, liver, etc)	oncogene, cell growth
hsa-miR-103a-3p	2531	3552	embryonic stem cells, many tissues/cells	various cancers (endometrial, neuroblastoma, colorectal, breast, liver, etc)	oncogene, cell growth
hsa-miR-103b	2532	3553	Many tissues/cells	various cancers (endometrial, neuroblastoma, colorectal, breast, liver, etc)	oncogene, cell growth
hsa-miR-105-3p	2533	3554	pancreatic cells		
hsa-miR-105-5p	2534	3555	pancreatic cells		
hsa-miR-106a-3p	2535	3556	osteogenic cells	osteocarcoma, other cancers	cell differentiation
hsa-miR-106a-5p	2536	3557	osteogenic cells	osteocarcoma, other cancers	cell differentiation
hsa-miR-106b-3p	2537	3558	embryonic stem cells	various cancers (non-small lung cancer, gastric cancer, HCC, gliomas, etc)	oncogene
hsa-miR-106b-5p	2538	3559	embryonic stem cells	various cancers (non-small lung	oncogene

				cancer, gastric cancer, HCC, gliomas, etc)	
hsa-miR-107	2539	3560	many tissues, brain hepatocytes/liver	breast cancer, pituitary adenoma, obesity/diabetes	
hsa-miR-10a-3p	2540	3561	hematopoeitic cells	acute myeoid leukemia	oncogene, cell growth
hsa-miR-10a-5p	2541	3562	hematopoeitic cells	acute myeoid leukemia	oncogene, cell growth
hsa-miR-10b-3p	2542	3563	multiple tissues and cells	various cancers (breast, ovarian, glioblastoma, pancreatc ductal adenocarcinoma, gastric, etc)	oncogene
hsa-miR-10b-5p	2543	3564	multiple tissues and cells	various cancers (breast, ovarian, glioblastoma, pancreatc ductal adenocarcinoma, gastric, etc)	oncogene
hsa-miR-1178-3p	2544	3565		osteocarcoma	
hsa-miR-1178-5p	2545	3566		osteocarcoma	
hsa-miR-1179	2546	3567		osteocarcoma	
hsa-miR-1180	2547	3568	discovered in sarcoma, no expression data		
hsa-miR-1181	2548	3569		downregulated in ovarian cancer cells, associated with HCV infection in hepatocytes	
hsa-miR-1182	2549	3570	placenta		
hsa-miR-1183	2550	3571		associated with rectal cancer	
hsa-miR-1184	2551	3572	Hematopoietic cells	downregulated in oral leukoplakia (OLK)	
hsa-miR-1185-1-3p	2552	3573	placenta		
hsa-miR-1185-2-3p	2553	3574	placenta		
hsa-miR-1185-5p	2554	3575	placenta		
hsa-miR-1193	2555	3576		melanoma	
hsa-miR-1197	2556	3577		neublastoma	
hsa-miR-1200	2557	3578		chronic lynphocytic leukemia	
hsa-miR-1202	2558	3579		chronic lynphocytic	

				leukemia, downregulated in ovarian cancer cells	
hsa-miR-1203	2559	3580		in the chromosome 8q24 region, cancer cells	
hsa-miR-1204	2560	3581		in the chromosome 8q24 region, cancer cells	
hsa-miR-1205	2561	3582		in the chromosome 8q24 region, cancer cells	
hsa-miR-1206	2562	3583		in the chromosome 8q24 region, cancer cells	
hsa-miR-1207-3p	2563	3584		in the chromosome 8q24 region, cancer cells	
hsa-miR-1207-5p	2564	3585		in the chromosome 8q24 region, cancer cells	
hsa-miR-1208	2565	3586		in the chromosome 8q24 region, cancer cells	
hsa-miR-122-3p	2566	3587	kidney, liver/hepatocytes	Renal Cell Carcinoma (RCC), cancer cells	lipid metabolism
hsa-miR-1224-3p	2567	3588		Lupus nephritis	
hsa-miR-1224-5p	2568	3589		rectal cancer	
hsa-miR-1225-3p	2569	3590		adrenal pheochromocytomas; upregulated in MITF KnockDown melanocytes	
hsa-miR-1225-5p	2570	3591		prostate cancer	
hsa-miR-122-5p	2571	3592	liver/hepatocytes	cancer cells	lipid metabolism
hsa-miR-1226-3p	2572	3593	discovered in a mirtron screening		
hsa-miR-1226-5p	2573	3594	discovered in a mirtron screening		
hsa-miR-1227-3p	2574	3595	cartilage/chondrocytes		
hsa-miR-1227-5p	2575	3596	cartilage/chondrocy		

			tes		
hsa-miR-1228-3p	2576	3597	liver(hepatocytes)	Hepatocellular carcinoma(HCC)	anti-apoptosis
hsa-miR-1228-5p	2577	3598	liver(hepatocytes)	Hepatocellular carcinoma(HCC)	anti-apoptosis
hsa-miR-1229-3p	2578	3599	discovered in a mirtron screening		
hsa-miR-1229-5p	2579	3600	discovered in a mirtron screening		
hsa-miR-1231	2580	3601		HCC	
hsa-miR-1233-1-5p	2581	3602	serum		
hsa-miR-1233-3p	2582	3603	serum		
hsa-miR-1234-3p	2583	3604	discovered in embryonic stem cell		
hsa-miR-1234-5p	2584	3605	discovered in embryonic stem cell		
hsa-miR-1236-3p	2585	3606	lymphatic endothelial cells		target to VEGFR-3
hsa-miR-1236-5p	2586	3607	lymphatic endothelial cells		target to VEGFR-3
hsa-miR-1237-3p	2587	3608	esophageal cell line KYSE-150R		
hsa-miR-1237-5p	2588	3609	esophageal cell line KYSE-150R		
hsa-miR-1238-3p	2589	3610		colorectal cancer	
hsa-miR-1238-5p	2590	3611		colorectal cancer	
hsa-miR-1243	2591	3612	discovered in embryonic stem cells		
hsa-miR-124-3p	2592	3613	brain, plasma (exosomal)	glioma	cell differentiation
hsa-miR-1244	2593	3614	discovered in embryonic stem cells		
hsa-miR-1245a	2594	3615	discovered in embryonic stem cells		
hsa-miR-1245b-3p	2595	3616	discovered in embryonic stem cells		
hsa-miR-1245b-5p	2596	3617	discovered in embryonic stem cells		
hsa-miR-124-5p	2597	3618	brain, Plasma (circulating)	upregulated in heart dysfunction, glioma	cell differentiation
hsa-miR-1246	2598	3619	embryonic stem cells, epithelial cells		
hsa-miR-1247-3p	2599	3620	embryoid body cells		
hsa-miR-1247-5p	2600	3621	embryoid body cells		

hsa-miR-1248	2601	3622			component of SnoRNAs
hsa-miR-1249	2602	3623	liver(hepatocytes)		
hsa-miR-1250	2603	3624	oligodendrocytes		
hsa-miR-1251	2604	3625	discovered in embryonic stem cells		
hsa-miR-1252	2605	3626	discovered in embryonic stem cells		
hsa-miR-1253	2606	3627	discovered in embryonic stem cells		
hsa-miR-1254	2607	3628	embryonic stem cells		
hsa-miR-1255a	2608	3629	discovered in embryonic stem cells		
hsa-miR-1255b-2-3p	2609	3630	discovered in embryonic stem cells		
hsa-miR-1255b-5p	2610	3631	discovered in embryonic stem cells		
hsa-miR-1256	2611	3632	discovered in embryonic stem cells	prostate cancer	
hsa-miR-1257	2612	3633	discovered in embryonic stem cells	liposarcoma (soft tissue sarcoma)	
hsa-miR-1258	2613	3634	discovered in embryonic stem cells	breast cancer and lung cancer	
hsa-miR-125a-3p	2614	3635	brain, hematopoietic cells	various cancer (prostate, HCC, etc)	cell proliferation and differentiation
hsa-miR-125a-5p	2615	3636	brain, hematopoietic cells	various cancer (prostate, HCC, etc)	cell proliferation and differentiation
hsa-miR-125b-1-3p	2616	3637	hematopoietic cells (monocytes), brain(neuron)	various cancer (prostate, HCC, etc)	oncogene, cell differentiation
hsa-miR-125b-2-3p	2617	3638	hematopoietic cells (monocytes), brain(neuron)	various cancer (prostate, HCC, etc)	oncogene, cell differentiation
hsa-miR-125b-5p	2618	3639	hematopoietic cells, brain (neuron)	various cancer (cutaneous T cell lymphoma, prostate, HCC, etc)	oncogene, cell differentiation
hsa-miR-1260a	2619	3640	periodontal tissue		
hsa-miR-1260b	2620	3641	periodontal tissue		
hsa-miR-1261	2621	3642	embryonic stem cells		
hsa-miR-1262	2622	3643	embryoid body		

			cells		
hsa-miR-1263	2623	3644	discovered in embryonic stem cells		
hsa-miR-126-3p	2624	3645	endothelial cells,lung	B-lieage ALL	angiogenesis
hsa-miR-1264	2625	3646	discovered in embryonic stem cells		
hsa-miR-1265	2626	3647	discovered in embryonic stem cells		
hsa-miR-126-5p	2627	3648	endothelial cells,lung	breast cancer, B-lieage ALL	angiogenesis
hsa-miR-1266	2628	3649	embryonic stem cells		
hsa-miR-1267	2629	3650	discovered in embryonic stem cells		
hsa-miR-1268a	2630	3651	embryonic stem cells		
hsa-miR-1268b	2631	3652	embryonic stem cells		
hsa-miR- 1269a	2632	3653	embryoid body cells		
hsa-miR- 1269b	2633	3654	embryoid body cells		
hsa-miR- 1270	2634	3655	discovered in embryonic stem cells		
hsa-miR- 127l-3p	2635	3656	brain	Hepatocellular carcinoma(HCC)	Suppress GPC-3 inHCC
hsa-miR- 127l-5p	2636	3657	brain	Hepatocellular carcinoma(HCC)	Suppress GPC-3 inHCC
hsa-miR- 1272	2637	3658	embryonic stem cells		
hsa-miR- 1273a	2638	3659	discovered in embryonic stem cells		
hsa-miR- 1273c	2639	3660		colorectal cancer	
hsa-miR- 1273d	2640	3661	discovered in embryonic stem cells		
hsa-miR- 1273e	2641	3662		solid tumor cells	
hsa-miR- 1273f	2642	3663		cervical cancer	
hsa-miR- 1273g-3p	2643	3664		cervical cancer	
hsa-miR- 1273g-5p	2644	3665		cervical cancer	
hsa-miR- 127-3p	2645	3666	lung, placenta		
hsa-miR- 1275	2646	3667	embryonic stem cells	gastric carcinoma	
hsa-miR- 127-5p	2647	3668	lung, placenta(islet)		
hsa-miR- 1276	2648	3669	discovered in embryonic stem cells		
hsa-miR- 1277-3p	2649	3670	embryoid body		

			cells		
hsa-miR-1277-5p	2650	3671	embryoid body cells		
hsa-miR-1278	2651	3672	discovered in embryonic stem cells		
hsa-miR-1279	2652	3673	monocytes		
hsa-miR-128	2653	3674	glioblast, brain	B-lieage ALL	target to neurofibromin 1i n neuron
hsa-miR-1281	2654	3675		muscle invasive bladder cancer	
hsa-miR-1282	2655	3676	discovered in embryonic stem cells		
hsa-miR-1283	2656	3677	placenta		
hsa-miR-1284	2657	3678		lung cancer	
hsa-miR-1285-3p	2658	3679		various cancer cells	inhibit P53 expression
hsa-miR-1285-5p	2659	3680		various cancer cells	inhibit P53 expression
hsa-miR-1286	2660	3681	smooth muscle	esophageal cancer	
hsa-miR-1287	2661	3682	embryoid body cells	breast cancer	
hsa-miR-1288	2662	3683	discovered in embryonic stem cells		
hsa-miR-1289	2663	3684	multiple cell types		
hsa-miR-1290	2664	3685	embryoid body cells	gastric carcinoma	
hsa-miR-1291	2665	3686	hepatocytes		component of SnoRNAs
hsa-miR-129-l-3p	2666	3687	multiple cell types	HCC cancer cells	
hsa-miR-1292-3p	2667	3688			
hsa-miR-129-2-3p	2668	3689	multiple cell types	various cancer cells	
hsa-miR-1292-5p	2669	3690			
hsa-miR-1293	2670	3691	discovered in embryonic stem cells		
hsa-miR-1294	2671	3692	discovered in embryonic stem cells		
hsa-miR-1295a	2672	3693		tumor cells (follicular lymphoma)	
hsa-miR-1295b-3p	2673	3694		tumor cells (follicular lymphoma)	
hsa-miR-1295b-5p	2674	3695		tumor cells (follicular lymphoma)	
hsa-miR-129-5p	2675	3696	liver(hepatocytes)	HCC, thyroid cancer	cell death in cancer cell
hsa-miR-1296	2676	3697		breast cancer	

hsa-miR-1297	2677	3698	discovered in embryonic stem cells		
hsa-miR-1298	2678	3699			
hsa-miR-1299	2679	3700	discovered in embryonic stem cells		
hsa-miR-1301	2680	3701		breast cancer	
hsa-miR-1302	2681	3702			
hsa-miR-1303	2682	3703	hepatocyte	colorectal cancer, liver cancer	
hsa-miR-1304-3p	2683	3704			dental development
hsa-miR-1304-5p	2684	3705			dental development
hsa-miR-1305	2685	3706	discovered in embryonic stem cells		
hsa-miR-1306-3p	2686	3707	discovered in embryonic stem cells		
hsa-miR-1306-5p	2687	3708	discovered in embryonic stem cells		
hsa-miR-1307-3p	2688	3709	discovered in embryonic stem cells		
hsa-miR-1307-5p	2689	3710	discovered in embryonic stem cells		
hsa-miR-130a-3p	2690	3711	lung, monocytes, vascular endothelial cells	various cancers (basal cell carcinoma, HCC,ovarian, etc), drug resistance	pro-angiogenic
hsa-miR-130a-5p	2691	3712	lung, monocytes, vascular endothelial cells	various cancers (basal cell carcinoma, HCC,ovarian, etc), drug resistance	pro-angiogenic
hsa-miR-130b-3p	2692	3713	Lung, epidermal cells(keratinocytes)	various cancers (gastric, rena cell carcinoma)	cell proiferation/sen escence
hsa-miR-130b-5p	2693	3714	Lung, epidermal cells(keratinocytes)	various cancers (gastric, rena cell carcinoma)	cell proiferation/sen escence
hsa-miR-1321	2694	3715		neuroblastoma	
hsa-miR-1322	2695	3716		neuroblastoma	
hsa-miR-1323	2696	3717	placenta	neuroblastoma	
hsa-miR-132-3p	2697	3718	Brain(neuron), immune cells		
hsa-miR-1324	2698	3719		neuroblastoma	
hsa-miR-132-5p	2699	3720	brain(neuron),		

			immune cells		
hsa-miR-133a	2700	3721	muscle, heart, epithelial cells (lung)	heart failure, esophageal cancer	myogenesis
hsa-miR-133b	2701	3722	muscle, heart, epithelial cells (lung)	heart failure, esophageal cancer	myogenesis
hsa-miR-134	2702	3723	lung (epithelial)	non-samll cell lung cancer, pulmonary embolism	
hsa-miR-1343	2703	3724		breast cancer cells	
hsa-miR-135a-3p	2704	3725	brain, other tissues	various cancer cells (lung, breast, colorectal, HCC, etc)	tumor suppressor
hsa-miR-135a-5p	2705	3726	brain, other tissues	various cancer cells (lung, breast, colorectal, HCC, etc)	tumor suppressor
hsa-miR-135b-3p	2706	3727	brain, placenta, other tissues	various cancers (gastric, mammary,neurobl astomas, pancreatic, etc)	
hsa-miR-135b-5p	2707	3728	brain, placenta, other tissues	various cancers (gastric, mammary,neurobl astomas, pancreatic, etc)	
hsa-miR-136-3p	2708	3729	stem cells, placenta	glioma	tumor suppressor
hsa-miR-136-5p	2709	3730	stem cells, placenta	glioma	tumor suppressor
hsa-miR-137	2710	3731	brain	various cancers (glioblastoma, breast, gastric etc), Alzheimer's disease	inhibiting cancer cell proliferation and migration
hsa-miR-138-1-3p	2711	3732	stem cells, epidermal cells(keratinocytes)	arious cancer cells, downregulated in HCC	cell proliferation/sen escence
hsa-miR-138-2-3p	2712	3733	stem cells	arious cancer cells, downregulated in HCC	
hsa-miR-138-5p	2713	3734	stem cells	arious cancer cells, downregulated in HCC	
hsa-miR-139-3p	2714	3735	hematocytes, brain	various cancer cells (colorectal, gastric, ovarian)	repress cancer metastasis
hsa-miR-139-5p	2715	3736	hematocytes, brain	various cancer	repress cancer

				cells (colorectal, gastric, ovarian)	metastasis
hsa-miR-140-3p	2716	3737	airway smooth muscle	Virus infection, cancers	
hsa-miR-140-5p	2717	3738	cartilage (chondrocytes)	csncers	
hsa-miR-141-3p	2718	3739	Many tissues/cells	various cancer cells (HCC, prostate, kidney, etc)	cell differentiation
hsa-miR-141-5p	2719	3740	Many tissues/cells	various cancer cells (HCC, prostate, kidney, etc)	cell differentiation
hsa-miR-142-3p	2720	3741	meyloid cells, hematopoiesis, APC cells		immune response
hsa-miR-142-5p	2721	3742	meyloid cells, hematopoiesis, APC cells		immune response
hsa-miR-143-3p	2722	3743	vascular smooth muscle	pre-B-cell acute lymphocytic leukemia , virus infection	
hsa-miR-143-5p	2723	3744	vascular smooth muscle, T-cells	virus infection	
hsa-miR-144-3p	2724	3745	erythroid	various cancers (lung, colorectal, etc)	cell differentiation
hsa-miR-144-5p	2725	3746	erythroid	various cancers (lung, colorectal, etc)	cell differentiation
hsa-miR-145-3p	2726	3747	kidney, cartilage, vascular smooth muscle	T-cell lupus	tumor suppressor
hsa-miR-145-5p	2727	3748	kidney, cartilage, vascular smooth muscle	T-cell lupus	tumor suppressor
hsa-miR-1468	2728	3749		lung cancer	
hsa-miR-1469	2729	3750		tumor cell(follicular lymphoma), rectal cancer	
hsa-miR-146a-3p	2730	3751	immune cells, hematopoiesis	various cancers, endotoxin tolerance	
hsa-miR-146a-5p	2731	3752	immune cells, hematopoiesis	various cancers, endotoxin tolerance	
hsa-miR-146b-3p	2732	3753	immune cells	various cancers	
hsa-miR-146b-5p	2733	3754	Embryonic stem cells	various cancers (glioma)	tumor invation, migration
hsa-miR-1470	2734	3755			
hsa-miR-1471	2735	3756		tumor cell(follicular	

				lymphoma), rectal cancer	
hsa-miR-147a	2736	3757	Macrophage	inflammatory response	
hsa-miR-147b	2737	3758	Macrophage	inflammatory response	
hsa-miR-148a-3p	2738	3759	hematopoietic cells	CLL, T-lineage ALL	
hsa-miR-148a-5p	2739	3760	hematopoietic cells	CLL, T-lineage ALL	
hsa-miR-148b-3p	2740	3761	neuron		
hsa-miR-148b-5p	2741	3762	neuron		
hsa-miR-149-3p	2742	3763	heart, brain	various cancers (glioma, colorectal, gastric, etc)	
hsa-miR-149-5p	2743	3764	heart, brain	various cancers (glioma, colorectal, gastric, etc)	
hsa-miR-150-3p	2744	3765	hematopoietic cells (lymphoid)	circulating plasma (acute myeloid leukemia)	
hsa-miR-150-5p	2745	3766	hematopoietic cells (lymphoid)	circulating plasma (acute myeloid leukemia)	
hsa-miR-151a-3p	2746	3767	neuron, fetal liver		
hsa-miR-151a-5p	2747	3768	neuron, fetal liver		
hsa-miR-151b	2748	3769	immune cells (B-cells)		
hsa-miR-152	2749	3770	liver		
hsa-miR-153	2750	3771	brain		
hsa-miR-1537	2751	3772			
hsa-miR-1538	2752	3773	blood	Cancer cells	
hsa-miR-1539	2753	3774	esophageal cell line KYSE-150R		
hsa-miR-154-3p	2754	3775	embryonic stem cells		
hsa-miR-154-5p	2755	3776	embryonic stem cells		
hsa-miR-155-3p	2756	3777	T/B cells, monocytes,breast	various cancers (CLL, B cell lymphoma, breast, lung, ovarian, cervical, colorectal, prostate)	
hsa-miR-155-5p	2757	3778	T/B cells, monocytes,breast	various cancers (CLL, B cell lymphoma, breast, lung, ovarian, cervical, colorectal, prostate)	
hsa-miR-1587	2758	3779	identified in B-cells		

hsa-miR-15a-3p	2759	3780	blood, lymphocyte, hematopoietic tissues (spleen)		cell cycle, proliferation
hsa-miR- 15a-5p	2760	3781	blood, lymphocyte, hematopoietic tissues (spleen)		cell cycle, proliferation
hsa-miR-1 5b-3p	2761	3782	blood, lymphocyte, hematopoietic tissues (spleen)		cell cycle, proliferation
hsa-miR-15b-5p	2762	3783	blood, lymphocyte, hematopoietic tissues (spleen)		cell cycle, proliferation
hsa-miR- 16- l-3p	2763	3784	embryonic stem cells, blood, hematopoietic tissues (spleen)		
hsa-miR-1 6-2-3p	2764	3785	blood, lymphocyte, hematopoietic tissues (spleen)		
hsa-miR- 16-5p	2765	3786	Many tissues, blood		
hsa-miR- 17-3p	2766	3787	embryonic stem cells, endothelial cells,		tumor angiogenesis
hsa-miR-1 7-5p	2767	3788	endothelial cells, kidney, breast;		tumor angiogenesis
hsa-miR- 18 1a-2-3p	2768	3789	glioblast, stem cells		
hsa-miR-1 81a-3p	2769	3790	glioblast, myeloid cells, Embryonic stem cells		
hsa-miR- 18 la-5p	2770	379 1	glioblast, myeloid cells, Embryonic stem cells		
hsa-miR- 18 lb-3p	2771	3792	glioblast, Embryonic stem cells , epidermal (keratinocytes)		cell proiferation/sen escence
hsa-miR- 18 lb-5p	2772	3793	glioblast, Embryonic stem cells , epidermal (keratinocytes)		cell proiferation/sen escence
hsa-miR- 18 lc-3p	2773	3794	brain, stem cells/progenitor	variou cance cells (gliobasltoma, basal cell carcinoma, prostate)	cell differentiation
hsa-miR- 18 lc-5p	2774	3795	brain, stem cells/progenitor	variou cance cells (gliobasltoma, basal cell carcinoma, prostate)	cell differentiation
hsa-miR- 18 1d	2775	3796	glia cells		
hsa-miR- 182-3p	2776	3797	immune cells	autoimmune	immune response
hsa-miR- 1825	2777	3798	discovered in a MiRDeep screening		

hsa-miR-182-5p	2778	3799	lung, immune cells	autoimmune	immune response
hsa-miR-1827	2779	3800		small cell lung cancer	
hsa-miR-183-3p	2780	3801	brain		
hsa-miR-183-5p	2781	3802	brain		
hsa-miR-184	2782	3803	blood, tongue, pancreas (islet)		
hsa-miR-185-3p	2783	3804			
hsa-miR-185-5p	2784	3805			
hsa-miR-186-3p	2785	3806	osteoblasts, heart	various cancer cells	
hsa-miR-186-5p	2786	3807	osteoblasts, heart	various cancer cells	
hsa-miR-187-3p	2787	3808		thyroid tumor	
hsa-miR-187-5p	2788	3809		thyroid tumor	
hsa-miR-188-3p	2789	3810	irway smooth muscle, central nervous system		
hsa-miR-188-5p	2790	3811	irway smooth muscle, central nervous system		
hsa-miR-18a-3p	2791	3812	endothelial cells, lung		
hsa-miR-18a-5p	2792	3813	endothelial cells, lung		
hsa-miR-18b-3p	2793	3814	lung		
hsa-miR-18b-5p	2794	3815	lung		
hsa-miR-1908	2795	3816		breast cancer	
hsa-miR-1909-3p	2796	3817		rectal cancer	
hsa-miR-1909-5p	2797	3818		rectal cancer	
hsa-miR-190a	2798	3819	brain		
hsa-miR-190b	2799	3820	brain		
hsa-miR-1910	2800	3821	embryonic stem cells		
hsa-miR-1911-3p	2801	3822	embryonic stem cells, neural precursor		
hsa-miR-1911-5p	2802	3823	embryonic stem cells, neural precursor		
hsa-miR-1912	2803	3824	embryonic stem cells, neural precursor		
hsa-miR-1913	2804	3825	embryonic stem cells		
hsa-miR-191-3p	2805	3826		chroninc lymphocyte leukimia, B-lieage ALL	
hsa-miR-1914-3p	2806	3827	embryonic stem cells		
hsa-miR-1914-5p	2807	3828	embryonic stem cells		

hsa-miR-1915-3p	2808	3829	embryonic stem cells		
hsa-miR-1915-5p	2809	3830	embryonic stem cells		
hsa-miR-191-5p	2810	3831		chroninc lymphocyte leukimia, B-lieage ALL	
hsa-miR-192-3p	2811	3832	kidney		
hsa-miR-192-5p	2812	3833	kidney		
hsa-miR-193a-3p	2813	3834	many tissues/cells	various cancer cells (lung, osteoblastoma, ALL, follicular lymphoma, etc)	tumor suppressor, proliferation
hsa-miR-193a-5p	2814	3835	many tissues/cells	various cancer cells (lung, osteoblastoma, ALL, follicular lymphoma, etc)	tumor suppressor, proliferation
hsa-miR-193b-3p	2815	3836	many tissues/cells, semen	arious cancer cells (prostate, breast, melanoma, myeloma, non small cell lung, etc)follicular lymphoma)	tumor suppressor
hsa-miR-193b-5p	2816	3837	many tissues/cells, semen	arious cancer cells (prostate, breast, melanoma, myeloma, non small cell lung, etc)follicular lymphoma)	tumor suppressor
hsa-miR-194-3p	2817	3838	kidney,liver	various cancers	
hsa-miR-194-5p	2818	3839	kidney,liver	various cancers	
hsa-miR-195-3p	2819	3840	breast, pancreas (islet)		
hsa-miR-195-5p	2820	3841	breast, pancreas (islet)		
hsa-miR-196a-3p	2821	3842	pancreatic cells,endometrial tissues, mesenchymal stem cells	various cancer cells (pancreatic, osteosarcoma, endometrial, AML etc)	oncogenic, tumor suppressor
hsa-miR- 196a-5p	2822	3843	pancreatic cells,endometrial tissues, mesenchymal stem cells	various cancer cells (pancreatic, osteosarcoma, endometrial, AML etc)	oncogenic, tumor suppressor
hsa-miR- 196b-3p	2823	3844	endometrial tissues	glioblastoma	apoptosis
hsa-miR- 196b-5p	2824	3845	endometrial tissues	glioblastoma	apoptosis
hsa-miR- 1972	2825	3846		acute lymphoblastic leukemia	

hsa-miR-1973	2826	3847		acute lymphoblastic leukemia	
hsa-miR-197-3p	2827	3848	blood (myeloid), other tissues/cells	various cancers (thyroid tumor, leukemia, etc)	
hsa-miR-197-5p	2828	3849	blood (myeloid), other tissues/cells	various cancers (thyroid tumor, leukemia, etc)	
hsa-miR-1976	2829	3850		acute lymphoblastic leukemia	
hsa-miR-198	2830	385 1	central nevous **system(CNS)**		
hsa-miR- 199a-3p	283 1	3852	liver, embryoid body cells, cardiomyocytes		
hsa-miR- 199a-5p	2832	3853	liver, cardiomyocytes		
hsa-miR- 199b-3p	2833	3854	liver, osteoblast	various cancers	osteogenesis
hsa-miR- 199b-5p	2834	3855	liver, osteoblast	various cancers	osteogenesis
hsa-miR- 19a-3p	2835	3856	endothelial cells		tumor angiogenesis
hsa-miR- 19a-5p	2836	3857	endothelial cells		tumor angiogenesis
hsa-miR- 19b-l-5p	2837	3858	endothelial cells		tumor angiogenesis
hsa-miR- 19b-2-5p	2838	3859	endothelial cells		tumor angiogenesis
hsa-miR- 19b-3p	2839	3860	endothelial cells		tumor angiogenesis
hsa-miR-200a-3p	2840	3861	epithelial cells, many other tissues	various cancers (breast, cervical, bladder, etc)	tumor progression and metastasis
hsa-miR-200a-5p	2841	3862	epithelial cells, many other tissues	various cancers (breast, cervical, bladder, etc)	tumor progression and metastasis
hsa-miR-200b-3p	2842	3863	epithelial cells, many other tissues		tumor progression and metastasis
hsa-miR-200b-5p	2843	3864	epithelial cells, many other tissues		tumor progression and metastasis
hsa-miR-200c-3p	2844	3865	epithelial cells, many other tissues, embryonic stem cells		tumor progression and metastasis
hsa-miR-200c-5p	2845	3866	epithelial cells, many other tissues, embryonic stem cells		tumor progression and metastasis
hsa-miR-202-3p	2846	3867	blood	lymphomagenesis , other cancers	
hsa-miR-202-5p	2847	3868	blood	lymphomagenesis , other cancers	

hsa-miR-203a	2848	3869	skin (epithelium)	psoriasis, autoimmune	
hsa-miR-203b-3p	2849	3870	skin specific (epithelium)	psoriasis, autoimmune	
hsa-miR-203b-5p	2850	3871	skin specific (epithelium)	psoriasis, autoimmune	
hsa-miR-204-3p	2851	3872	adipose, other tissues/cells. kidney	various cancers	tumor metastasis
hsa-miR-204-5p	2852	3873	adipose, other tissues/cells, kidney	various cancers	tumor metastasis
hsa-miR-2052	2853	3874			
hsa-miR-2053	2854	3875			
hsa-miR-205-3p	2855	3876	blood(plasma)	various cancer cells (breast, glioma, melanoma, endometrial, etc)	
hsa-miR-2054	2856	3877			
hsa-miR-205-5p	2857	3878	blood(plasma)	various cancer cells (breast, glioma, melanoma, endometrial, etc)	
hsa-miR-206	2858	3879	muscle (cardiac and skeletal)		myogenesis
hsa-miR-208a	2859	3880	heart(cardiomyocyte), muscle	cardiac defects	
hsa-miR-208b	2860	3881	heart(cardiomyocyte), muscle	cardiac defects	
hsa-miR-20a-3p	2861	3882	endothelial cells, kidney, osteogenic cells		
hsa-miR-20a-5p	2862	3883	endothelial cells, kidney, osteogenic cells		
hsa-miR-20b-3p	2863	3884	osteogenic cells		
hsa-miR-20b-5p	2864	3885	osteogenic cells		
hsa-miR-210	2865	3886	kidney, heart, vascular endothelial cells	RCC, B-cell lymphocytes	angiogenesis
hsa-miR-2110	2866	3887		rectal cancer	
hsa-miR-2113	2867	3888	embryonic stem cells		
hsa-miR-211-3p	2868	3889	melanocytes	melanoma and other cancers	
hsa-miR-2114-3p	2869	3890	ovary, female reproductuve tract		
hsa-miR-2114-5p	2870	3891	ovary, female reproductuve tract		
hsa-miR-2115-3p	2871	3892	female reproductive tract	ovarian cancer	
hsa-miR-2115-5p	2872	3893	female reproductive tract	ovarian cancer	
hsa-miR-211-5p	2873	3894	melanocytes	melanoma and other cancers	

hsa-miR-2116-3p	2874	3895		live cancer(hepatocytes) and ovarian cancer	
hsa-miR-2116-5p	2875	3896		live cancer(hepatocytes) and ovarian cancer	
hsa-miR-2117	2876	3897		ovarian cancer	
hsa-miR-212-3p	2877	3898	brain(neuron), spleen	lymphoma	
hsa-miR-212-5p	2878	3899	brain(neuron), spleen	lymphoma	
hsa-miR-21-3p	2879	3900	glioblast, Blood (meyloid cells), liver, vascular endothelial cells	autoimmune, heart diseases, cancers	
hsa-miR-214-3p	2880	3901	immune cerlls, pancreas	varioua cancers (melanoma, pancreatic, ovarian)	immune response
hsa-miR-214-5p	2881	3902	immune cells, pancreas	varioua cancers (melanoma, pancreatic, ovarian)	immune response
hsa-miR-215	2882	3903	many tissues/cells	various cancers (renal, colon, osteosarcoma)	cell cycle arrest/p53 inducible
hsa-miR-21-5p	2883	3904	blood (myeloid cells), liver, endothelial cells	autoimmune, heart diseases, cancers	
hsa-miR-216a-3p	2884	3905	kidney, pancreas		
hsa-miR-216a-5p	2885	3906	kidney, pancreas		
hsa-miR-216b	2886	3907		cancers	senescence
hsa-miR-217	2887	3908	endothelial cells	various cancer cells (pancreas, kidney, breast)	
hsa-miR-218-1-3p	2888	3909	endothelial cells	various cancer cells (gastric tumor, bladder, cervical, etc)	
hsa-miR-218-2-3p	2889	3910		various cancer cells (gastric tumor, bladder, cervical, etc)	
hsa-miR-218-5p	2890	3911		various cancer cells (gastric tumor, bladder, cervical, etc)	
hsa-miR-219-1-3p	2891	3912	brain, oligodendrocytes		
hsa-miR-219-2-3p	2892	3913	brain, oligodendrocytes		
hsa-miR-219-5p	2893	3914	brain, oligodendrocytes		

hsa-miR-221-3p	2894	3915	endothelial cells, immune cells	leukemia and other cancers	angiogenesis/vasculogenesis
hsa-miR-22 1-5p	2895	3916	endothelial cells, immune cells	leukemia and other cancers	angiogenesis/vasculogenesis
hsa-miR-222-3p	2896	3917	endothelial cells	various cancers	angiogenesis
hsa-miR-222-5p	2897	3918	endothelial cells	various cancers	angiogenesis
hsa-miR-223-3p	2898	3919	meyloid cells	leukemia	
hsa-miR-223-5p	2899	3920	meyloid cells	leukemia	
hsa-miR-22-3p	2900	3921	many tissues/cells	various cancers	tumorigenesis
hsa-miR-224-3p	2901	3922	blood(plasma), ovary	cancers and inflammation	
hsa-miR-224-5p	2902	3923	blood(plasma), ovary	cancers and inflammation	
hsa-miR-22-5p	2903	3924	many tissues/cells	Various cancers	tumorigenesis
hsa-miR-2276	2904	3925		breast cancer	
hsa-miR-2277-3p	2905	3926	female reproductive tract		
hsa-miR-2277-5p	2906	3927	female reproductive tract		
hsa-miR-2278	2907	3928		breast cancer	
hsa-miR-2355-3p	2908	3929	embryonic stem cells		
hsa-miR-2355-5p	2909	3930	embryonic stem cells		
hsa-miR-2392	2910	3931	identified in B-cells		
hsa-miR-23a-3p	2911	3932	brain(astrocyte), endothelial cells, blood(erythroid)	Cancers	
hsa-miR-23a-5p	2912	3933	brain(astrocyte), endothelial cells, blood(erythroid)	cancers	
hsa-miR-23b-3p	2913	3934	blood, meyloid cells	cancers (renal cancer, glioblastoma, prostate, etc) and autoimmune	
hsa-miR-23b-5p	2914	3935	blood, meyloid cells	cancers(glioblastoma, prostate, etc) and autoimmune	
hsa-miR-23c	2915	3936		cervical cancer	
hsa-miR-24-l-5p	2916	3937	lung, meyloid cells		
hsa-miR-24-2-5p	2917	3938	lung, meyloid cells		
hsa-miR-24-3p	2918	3939	lung, meyloid cells		
hsa-miR-2467-3p	2919	3940		breast cancer	
hsa-miR-2467-5p	2920	3941		breast cancer	
hsa-miR-25-3p	2921	3942	embryonic stem cells, airway smooth muscle		
hsa-miR-25-5p	2922	3943	embryonic stem cells, airway smooth muscle		
hsa-miR-268 1-3p	2923	3944		breast cancer	
hsa-miR-268 1-5p	2924	3945		breast cancer	
hsa-miR-2682-3p	2925	3946			

hsa-miR-2682-5p	2926	3947			
hsa-miR-26a- 1-3p	2927	3948	embryonic stem cells, blood , other tissues	CLL and other cancers	cell cycle and differentiation
hsa-miR-26a-2-3p	2928	3949	blood , other tissues	CLL and other cancers	cell cycle and differentiation
hsa-miR-26a-5p	2929	3950	blood , other tissues	CLL and other cancers	cell cycle and differentiation
hsa-miR-26b-3p	2930	3951	hematopoietic cells		
hsa-miR-26b-5p	2931	3952	hematopoietic cells		
hsa-miR-27a-3p	2932	3953	meyloid cells	various cancer cells	
hsa-miR-27a-5p	2933	3954	meyloid cells	various cancer cells	
hsa-miR-27b-3p	2934	3955	meyloid cells, vascular endothelial cells	various cancer cells	pro-angiogenic
hsa-miR-27b-5p	2935	3956	meyloid cells, vascular endothelial cells	various cancer cells	pro-angiogenic
hsa-miR-28-3p	2936	3957	blood(immune cells)	B/T cell lymphoma	
hsa-miR-28-5p	2937	3958	blood(immune cells)	B/T cell lymphoma	
hsa-miR-2861	2938	3959	osteoblasts	basal cell carcinoma	
hsa-miR-2909	2939	3960	T-Lymphocytes		
hsa-miR-296-3p	2940	3961	kidney, heart,lung, entothelial cells		angiogenesis
hsa-miR-2964a-3p	2941	3962			
hsa-miR-2964a-5p	2942	3963			
hsa-miR-296-5p	2943	3964	lung, liver, endothelial cells		angiogenesis
hsa-miR-297	2944	3965	oocyte and prostate		
hsa-miR-298	2945	3966		breast cancer	
hsa-miR-299-3p	2946	3967		myeloid leukaemia, hepatoma, breast cancer	
hsa-miR-299-5p	2947	3968		myeloid leukaemia, hepatoma, breast cancer	
hsa-miR-29a-3p	2948	3969	immuno system	CLL, other cancers, neurodegenative disease	tumor suppression, immune modulation
hsa-miR-29a-5p	2949	3970	immuno system	CLL, other cancers, neurodegenative disease	tumor suppression, immune modulation
hsa-miR-29b-l-5p	2950	3971	immuno system	CLL, other cancers, neurodegenative disease	tumor suppression, immune modulation

hsa-miR-29b-2-5p	2951	3972	immuno system	CLL, other cancers	tumor suppression, immune modulation
hsa-miR-29b-3p	2952	3973	immuno system	CLL, other cancers	tumor suppression, immune modulation
hsa-miR-29c-3p	2953	3974	immuno system	CLL, other cancers	tumor suppression, immune modulation
hsa-miR-29c-5p	2954	3975	immuno system	CLL, other cancers	tumor suppression, immune modulation
hsa-miR-300	2955	3976	osteoblast	Bladder cancer	
hsa-miR-301a-3p	2956	3977	embryonic stem cells		
hsa-miR-301a-5p	2957	3978	embryonic stem cells		
hsa-miR-301b	2958	3979		esophageal adenocarcinoma, colonic cancer	
hsa-miR-302a-3p	2959	3980	embryonic stem cells, lipid metabolism		lipid metabolism
hsa-miR-302a-5p	2960	3981	embryonic stem cells, lipid metabolism		lipid metabolism
hsa-miR-302b-3p	2961	3982	embryonic stem cells		
hsa-miR-302b-5p	2962	3983	embryonic stem cells		
hsa-miR-302c-3p	2963	3984	embryonic stem cells		
hsa-miR-302c-5p	2964	3985	embryonic stem cells		
hsa-miR-302d-3p	2965	3986	embryonic stem cells		
hsa-miR-302d-5p	2966	3987	embryonic stem cells		
hsa-miR-302e	2967	3988	embryoid body cells		
hsa-miR-302f	2968	3989		gastric cancer	
hsa-miR-3064-3p	2969	3990			
hsa-miR-3064-5p	2970	3991			
hsa-miR-3065-3p	2971	3992	oligodendrocytes	anti-virus response	
hsa-miR-3065-5p	2972	3993	oligodendrocytes	solid tumors	
hsa-miR-3074-3p	2973	3994		various cancer(melanoma, breast)	
hsa-miR-3074-5p	2974	3995		various cancer(melanoma,	

				breast)	
hsa-miR-30a-3p	2975	3996	kidney, pancreatic cells	various cancers	autophagy
hsa-miR-30a-5p	2976	3997	CNS(prefrontal cortex), other tissues	glioma, colon carcinoma	autophagy
hsa-miR-30b-3p	2977	3998	kidney, adipose, CNS(prefrontal cortex)		
hsa-miR-30b-5p	2978	3999	kidney, adipose, CNS(prefrontal cortex)		
hsa-miR-30c-1-3p	2979	4000	kidney, adipose, CNS(prefrontal cortex)		
hsa-miR-30c-2-3p	2980	4001	kidney, adipose, CNS(prefrontal cortex)		
hsa-miR-30c-5p	2981	4002	kidney, adipose, CNS(prefrontal cortex)		
hsa-miR-30d-3p	2982	4003	CNS (prefrontal cortex		
hsa-miR-30d-5p	2983	4004	CNS (prefrontal cortex, embryoid body cells		
hsa-miR-30e-3p	2984	4005	myeloid cells, glia cells		
hsa-miR-30e-5p	2985	4006	myeloid cells, glia cells		
hsa-miR-3115	2986	4007		various cancer (melanoma, breast tumor)	
hsa-miR-3116	2987	4008	discovered in the melanoma miRNAome		
hsa-miR-3117-3p	2988	4009	discovered in the melanoma miRNAome		
hsa-miR-3117-5p	2989	4010	discovered in the melanoma miRNAome		
hsa-miR-3118	2990	4011	discovered in the melanoma miRNAome		
hsa-miR-3119	2991	4012	discovered in the melanoma miRNAome		
hsa-miR-3120-3p	2992	4013	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3120-5p	2993	4014	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3121-3p	2994	4015	discovered in the	breast tumor	

			melanoma miRNAome		
hsa-miR-3 12 1-5p	2995	4016	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 122	2996	4017	discovered in the melanoma miRNAome		
hsa-miR-3 123	2997	401 8	discovered in the melanoma miRNAome		
hsa-miR-3 124-3p	2998	4019	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 124-5p	2999	4020	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 125	3000	4021	discovered in the melanoma miRNAome		
hsa-miR-3 126-3p	3001	4022	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 126-5p	3002	4023	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 127-3p	3003	4024	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 127-5p	3004	4025	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 128	3005	4026	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 129-3p	3006	4027	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 129-5p	3007	4028	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 130-3p	3008	4029	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 130-5p	3009	4030	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 13 1	3010	403 1	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 132	301 1	4032	discovered in the melanoma miRNAome		
hsa-miR-3 133	3012	4033	discovered in the melanoma		

			miRNAome		
hsa-miR-3 134	3013	4034	discovered in the melanoma miRNAome		
hsa-miR-3 135a	3014	4035	discovered in the melanoma miRNAome		
hsa-miR-3 135b	3015	4036	discovered in B cells		
hsa-miR-3 136-3p	3016	4037	discovered in the melanoma miRNAome	lymphoblastic leukaemia and breast tumor	
hsa-miR-3 136-5p	3017	4038	discovered in the melanoma miRNAome	lymphoblastic leukaemia and breast tumor	
hsa-miR-3 137	301 8	4039	discovered in the melanoma miRNAome		
hsa-miR-3 138	3019	4040	discovered in the melanoma miRNAome, ovary		
hsa-miR-3 139	3020	4041	discovered in the melanoma miRNAome		
hsa-miR-3 1-3p	3021	4042			
hsa-miR-3 140-3p	3022	4043	discovered in the melanoma miRNAome, ovary	lymphoblastic leukaemia and breast tumor	
hsa-miR-3 140-5p	3023	4044	discovered in the melanoma miRNAome, ovary	lymphoblastic leukaemia and breast tumor	
hsa-miR-3 141	3024	4045	discovered in the melanoma miRNAome		
hsa-miR-3 142	3025	4046	discovered in the melanoma miRNAome; immune cells		
hsa-miR-3 143	3026	4047	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 144-3p	3027	4048	discovered in the melanoma miRNAome, ovary		
hsa-miR-3 144-5p	3028	4049	discovered in the melanoma miRNAome, ovary		
hsa-miR-3 145-3p	3029	4050	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 145-5p	3030	405 1	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 146	303 1	4052	discovered in the melanoma	breast tumor	

			miRNAome		
hsa-miR-3 147	3032	4053	discovered in the melanoma miRNAome		
hsa-miR-3 148	3033	4054	discovered in the melanoma miRNAome		
hsa-miR-3 149	3034	4055	discovered in the melanoma miRNAome, ovary		
hsa-miR-3 150a-3p	3035	4056	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 150a-5p	3036	4057	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 150b-3p	3037	4058	discovered in the melanoma miRNAome	breast tumor and lymphoblastic leukaemia	
hsa-miR-3 150b-5p	3038	4059	discovered in the melanoma miRNAome	breast tumor and lymphoblastic leukaemia	
hsa-miR-3 15 1	3039	4060	discovered in the melanoma miRNAome	lymphoblastic leukaemia	
hsa-miR-3 152-3p	3040	4061	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 152-5p	304 1	4062	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 153	3042	4063	discovered in the melanoma miRNAome		
hsa-miR-3 154	3043	4064	discovered in the melanoma miRNAome	lymphoblastic leukaemia	
hsa-miR-3 155a	3044	4065	discovered in the melanoma miRNAome		
hsa-miR-3 155b	3045	4066	discovered in B cells		
hsa-miR-3 156-3p	3046	4067	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 156-5p	3047	4068	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 157-3p	3048	4069	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 157-5p	3049	4070	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 158-3p	3050	4071	discovered in the	breast tumor	

			melanoma miRNAome, ovary		
hsa-miR-3 158-5p	305 1	4072	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 159	3052	4073	discovered in the melanoma miRNAome		
hsa-miR-3 1-5p	3053	4074		various cancer cells (breast, lung, prostate)	
hsa-miR-3 160-3p	3054	4075	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 160-5p	3055	4076	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 161	3056	4077	discovered in the melanoma miRNAome		
hsa-miR-3 162-3p	3057	4078	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 162-5p	3058	4079	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 163	3059	4080	discovered in the melanoma miRNAome		
hsa-miR-3 164	3060	408 1	discovered in the melanoma miRNAome		
hsa-miR-3 165	3061	4082	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 166	3062	4083	discovered in the melanoma miRNAome		
hsa-miR-3 167	3063	4084	discovered in the melanoma miRNAome, ovary		
hsa-miR-3 168	3064	4085	discovered in the melanoma miRNAome		
hsa-miR-3 169	3065	4086	discovered in the melanoma miRNAome		
hsa-miR-3 170	3066	4087	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 171	3067	4088	discovered in the melanoma miRNAome, ovary		
hsa-miR-3 173-3p	3068	4089	discovered in the melanoma	breast tumor	

			miRNAome		
hsa-miR-3 173-5p	3069	4090	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 174	3070	409 1	discovered in the melanoma miRNAome		
hsa-miR-3 175	3071	4092	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 176	3072	4093	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3 177-3p	3073	4094	discovered in the melanoma miRNAome	breast tumor and lymphoblastic leukaemia	
hsa-miR-3 177-5p	3074	4095	discovered in the melanoma miRNAome	breast tumor and lymphoblastic leukaemia	
hsa-miR-3 178	3075	4096	discovered in the melanoma miRNAome		
hsa-miR-3 179	3076	4097	discovered in the melanoma miRNAome		
hsa-miR-3 180	3077	4098	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3 180-3p	3078	4099	discovered in breast tunor		
hsa-miR-3 180-5p	3079	4 100	discovered in breast tumor		
hsa-miR-3 18 1	3080	4 10 1	discovered in the melanoma miRNAome		
hsa-miR-3 182	3081	4 102	discovered in the melanoma miRNAome		
hsa-miR-3 183	3082	4103	discovered in the melanoma miRNAome		
hsa-miR-3 184-3p	3083	4 104	discovered in the melanoma miRNAome		
hsa-miR-3 184-5p	3084	4105	discovered in the melanoma miRNAome		
hsa-miR-3 185	3085	4 106	discovered in the melanoma miRNAome		
hsa-miR-3 186-3p	3086	4107	discovered in the melanoma miRNAome, ovary		
hsa-miR-3 186-5p	3087	4108	discovered in the melanoma		

			miRNAome, ovary		
hsa-miR-3187-3p	3088	4109	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3187-5p	3089	4110	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3188	3090	4111	discovered in the melanoma miRNAome		
hsa-miR-3189-3p	3091	4112	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3189-5p	3092	4113	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3190-3p	3093	4114	discovered in the melanoma miRNAome	lymphoblastic leukaemia	
hsa-miR-3190-5p	3094	4115	discovered in the melanoma miRNAome	lymphoblastic leukaemia	
hsa-miR-3191-3p	3095	4116	discovered in the melanoma miRNAome		
hsa-miR-3191-5p	3096	4117	discovered in the melanoma miRNAome		
hsa-miR-3192	3097	4118	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3193	3098	4119	discovered in the melanoma miRNAome		
hsa-miR-3194-3p	3099	4120	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3194-5p	3100	4121	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3195	3101	4122	discovered in the melanoma miRNAome		
hsa-miR-3196	3102	4123		basal cell carcinoma	
hsa-miR-3197	3103	4124	discovered in the melanoma miRNAome		
hsa-miR-3198	3104	4125	discovered in the melanoma miRNAome	breast tumor	
hsa-miR-3199	3105	4126	discovered in the melanoma miRNAome		
hsa-miR-3200-3p	3106	4127	discovered in the	breast tumor	

			melanoma miRNAome, ovary		
hsa-miR-3200-5p	3107	4128	discovered in the melanoma miRNAome, ovary	breast tumor	
hsa-miR-3201	3108	4129	discovered in the melanoma miRNAome,		
hsa-miR-3202	3109	4130	discovered in the melanoma miRNAome,epithelial cell BEAS2B		
hsa-miR-320a	3110	4131	blood, heart(myocardiac)	colon cancer cells, heart disease	
hsa-miR-320b	3111	4132	central nevous system		
hsa-miR-320c	3112	4133	chondrocyte		cartilage metabolism
hsa-miR-320d	3113	4134		cancer stem cells	
hsa-miR-320e	3114	4135	neural cells		
hsa-miR-323a-3p	3115	4136	neurons	myeloid leukaemia, mudulla thyroid carcinoma	
hsa-miR-323a-5p	3116	4137	neurons	myeloid leukaemia, mudulla thyroid carcinoma	
hsa-miR-323b-3p	3117	4138		myeloid leukaemia	
hsa-miR-323b-5p	3118	4139		myeloid leukaemia	
hsa-miR-32-3p	3119	4140	blood, glia	various cancers (lung, kidney, prostate, etc), virus infection	
hsa-miR-324-3p	3120	4141	kidney		
hsa-miR-324-5p	3121	4142	neurons	tumor cells	
hsa-miR-325	3122	4143	neurons, placenta		
hsa-miR-32-5p	3123	4144	blood, glia	various cancers (lung, kidney, prostate, etc), virus infection	
hsa-miR-326	3124	4145	neurons	tumor cells	
hsa-miR-328	3125	4146	neuron, blood	tumor cells	
hsa-miR-329	3126	4147	brain and platele		
hsa-miR-330-3p	3127	4148		various cancers(prostate, glioblastoma, colorectal)	
hsa-miR-330-5p	3128	4149		various cancers(prostate, glioblastoma, colorectal)	

hsa-miR-331-3p	3129	4150		gastric cancer	
hsa-miR-331-5p	3130	4151	lymphocytes		
hsa-miR-335-3p	3131	4152	kidney, breast	RCC, multiple myeloma	
hsa-miR-335-5p	3132	4153	kidney, breast	RCC, multiple myeloma	
hsa-miR-337-3p	3133	4154	lung	gastric cancer	
hsa-miR-337-5p	3134	4155	lung		
hsa-miR-338-3p	3135	4156	epithelial cells, oligodendrocytes	gastric, rectal cancer cells, osteosarcoma	
hsa-miR-338-5p	3136	4157	oligodendrocytes	gastric cancer	
hsa-miR-339-3p	3137	4158	immune cell		
hsa-miR-339-5p	3138	4159	immune cell		
hsa-miR-33a-3p	3139	4160	pancreatic islet, lipid metabolism		lipid metabolism
hsa-miR-33a-5p	3140	4161	pancreatic islet, lipid metabolism		lipid metabolism
hsa-miR-33b-3p	3141	4162	lipid metabolism		lipid metabolism
hsa-miR-33b-5p	3142	4163	lipid metabolism		lipid metabolism
hsa-miR-340-3p	3143	4164		various cancers	
hsa-miR-340-5p	3144	4165	embryoid body cells		
hsa-miR-342-3p	3145	4166	brain, circulating plasma	multiple myeloma, other cancers	
hsa-miR-342-5p	3146	4167	circulating plasma	multiple myeloma, other cancers	
hsa-miR-345-3p	3147	4168	hematopoietic cells	follicular lymphoma, other cancers	
hsa-miR-345-5p	3148	4169	hematopoietic cells	follicular lymphoma, other cancers	
hsa-miR-346	3149	4170	immume cells	cancers and autoimmune	
hsa-miR-34a-3p	3150	4171	breast, meyloid cells, ciliated epithelial cells	gastric cancer, CLL, other	tumor suppressor, p53 inducible
hsa-miR-34a-5p	3151	4172	breast, meyloid cells, ciliated epithelial cells	gastric cancer, CLL, other	tumor suppressor, p53 inducible
hsa-miR-34b-3p	3152	4173	ciliated epithelial cells	various cancers	tumor suppressor, p53 inducible
hsa-miR-34b-5p	3153	4174	ciliated epithelial cells	various cancers	tumor suppressor, p53 inducible
hsa-miR-34c-3p	3154	4175	ciliated epithelial cells, placenta	various cancers	tumor suppressor, p53 inducible
hsa-miR-34c-5p	3155	4176	ciliated epithelial	various cancers	tumor

			cells, placenta		suppressor, p53 inducible
hsa-miR-3529-3p	3156	4177	discovered in breast tumor		
hsa-miR-3529-5p	3157	4178	discovered in breast tumor		
hsa-miR-3591-3p	3158	4179	discovered in breast tumor		
hsa-miR-3591-5p	3159	4180	discovered in breast tumor		
hsa-miR-3605-3p	3160	4181	discovered in reprodcutive_tracts		
hsa-miR-3605-5p	3161	4182	discovered in reprodcutive_tracts		
hsa-miR-3606-3p	3162	4183	discovered in cervical tumors		
hsa-miR-3606-5p	3163	4184	discovered in cervical tumors		
hsa-miR-3607-3p	3164	4185	discovered in cervical tumors		
hsa-miR-3607-5p	3165	4186	discovered in cervical tumors		
hsa-miR-3609	3166	4187	discovered in cervical tumors		
hsa-miR-3610	3167	4188	discovered in cervical tumors		
hsa-miR-361 1	3168	4189	discovered in cervical tumors		
hsa-miR-3612	3169	4190	discovered in cervical tumors		
hsa-miR-3613-3p	3170	4191	discovered in cervical tumors		
hsa-miR-3613-5p	3171	4192	discovered in cervical tumors		
hsa-miR-361-3p	3172	4193	blood, endothelial cells		
hsa-miR-3614-3p	3173	4194	discovered in cervical and breast tumors		
hsa-miR-3614-5p	3174	4195	discovered in cervical and breast tumors		
hsa-miR-361 5	3175	4196	discovered in cervical tumors		
hsa-miR-361-5p	3176	4197	endothelial cells		
hsa-miR-3616-3p	3177	4198	discovered in cervical tumors		
hsa-miR-3616-5p	3178	4199	discovered in cervical tumors		
hsa-miR-3617-3p	3179	4200	discovered in cervical tumors and psoriasis		
hsa-miR-3617-5p	3180	4201	discovered in cervical tumors and psoriasis		

hsa-miR-3618	3181	4202	discovered in cervical tumors		
hsa-miR-3619-3p	3182	4203	discovered in breast tumors		
hsa-miR-3619-5p	3183	4204	discovered in breast tumors		
hsa-miR-3620-3p	3184	4205	discovered in cervical tumors		
hsa-miR-3620-5p	3185	4206	discovered in cervical tumors		
hsa-miR-3621	3186	4207	discovered in cervical tumors		
hsa-miR-3622a-3p	3187	4208	discovered in breast tumors		
hsa-miR-3 622a-5p	3188	4209	discovered in breast tumors		
hsa-miR-3622b-3p	3189	4210	discovered in cervical tumors		
hsa-miR-3 622b-5p	3190	4211	discovered in cervical tumors		
hsa-miR-3 62-3p	3191	4212		melanoma	
hsa-miR-3 62-5p	3192	4213		melanoma	
hsa-miR-3 63-3p	3193	4214	kidney stem cell, blood cells		
hsa-miR-3 63-5p	3194	4215	kidney stem cell, blood cells		
hsa-miR-3 646	3195	4216	discovered in solid tumor		
hsa-miR-3648	3196	4217	discovered in solid tumor		
hsa-miR-3 649	3197	4218	discovered in solid tumor		
hsa-miR-3650	3198	4219	discovered in solid tumor		
hsa-miR-3 651	3199	4220	discovered in solid tumor		
hsa-miR-3 652	3200	4221	discovered in solid tumor		
hsa-miR-3653	3201	4222	discovered in solid tumor		
hsa-miR-3 654	3202	4223	discovered in solid tumor		
hsa-miR-3655	3203	4224	discovered in solid tumor		
hsa-miR-3656	3204	4225	discovered in solid tumor		
hsa-miR-3657	3205	4226	discovered in solid tumor		
hsa-miR-3 658	3206	4227	discovered in solid tumor		
hsa-miR-3 659	3207	4228	discovered in breast tumors		
hsa-miR-3 65a-3p	3208	4229		various cancer cells (Immune cells, lung, colon,	apoptosis

				endometriotic)	
hsa-miR-365a-5p	3209	4230		various cancer cells (Immune cells, lung, colon, endometriotic))	apoptosis
hsa-miR-365b-3p	3210	4231		various cancers (retinoblastoma,colon, endometriotic)	apoptosis
hsa-miR-365b-5p	3211	4232		various cancers (colon, endometriotic)	apoptosis
hsa-miR-3660	3212	4233	discovered in breast tumors		
hsa-miR-3661	3213	4234	discovered in breast tumors		
hsa-miR-3662	3214	4235			
hsa-miR-3663-3p	3215	4236			
hsa-miR-3663-5p	3216	4237			
hsa-miR-3664-3p	3217	4238	discovered in breast tumors		
hsa-miR-3664-5p	3218	4239	discovered in breast tumors		
hsa-miR-3665	3219	4240	brain		
hsa-miR-3666	3220	4241	brain		
hsa-miR-3667-3p	3221	4242	discovered in peripheral blood		
hsa-miR-3667-5p	3222	4243	discovered in peripheral blood		
hsa-miR-3668	3223	4244	discovered in peripheral blood		
hsa-miR-3669	3224	4245	discovered in peripheral blood		
hsa-miR-3670	3225	4246	discovered in peripheral blood		
hsa-miR-3671	3226	4247	discovered in peripheral blood		
hsa-miR-3672	3227	4248	discovered in peripheral blood		
hsa-miR-3673	3228	4249	discovered in peripheral blood		
hsa-miR-367-3p	3229	4250	embryonic stem cells		reprogramming
hsa-miR-3674	3230	4251	discovered in peripheral blood		
hsa-miR-3675-3p	3231	4252	discovered in peripheral blood		
hsa-miR-3675-5p	3232	4253	discovered in peripheral blood		
hsa-miR-367-5p	3233	4254	embryonic stem cells		reprogramming
hsa-miR-3676-3p	3234	4255	discovered in peripheral blood		
hsa-miR-3676-5p	3235	4256	discovered in peripheral blood		

hsa-miR-3677-3p	3236	4257	discovered in peripheral blood		
hsa-miR-3677-5p	3237	4258	discovered in peripheral blood		
hsa-miR-3678-3p	3238	4259	discovered in peripheral blood		
hsa-miR-3678-5p	3239	4260	discovered in peripheral blood		
hsa-miR-3679-3p	3240	4261	discovered in peripheral blood		
hsa-miR-3679-5p	3241	4262	discovered in peripheral blood		
hsa-miR-3680-3p	3242	4263	discovered in peripheral blood		
hsa-miR-3680-5p	3243	4264	discovered in peripheral blood		
hsa-miR-3681-3p	3244	4265	discovered in peripheral blood		
hsa-miR-3681-5p	3245	4266	discovered in peripheral blood		
hsa-miR-3682-3p	3246	4267	discovered in peripheral blood		
hsa-miR-3682-5p	3247	4268	discovered in peripheral blood		
hsa-miR-3683	3248	4269	discovered in peripheral blood		
hsa-miR-3684	3249	4270	discovered in peripheral blood		
hsa-miR-3685	3250	4271	discovered in peripheral blood		
hsa-miR-3686	3251	4272	discovered in peripheral blood		
hsa-miR-3687	3252	4273	discovered in peripheral blood		
hsa-miR-3688-3p	3253	4274	discovered in breast tumor		
hsa-miR-3688-5p	3254	4275	discovered in breast tumor		
hsa-miR-3689a-3p	3255	4276	discovered in female reproductuve tract		
hsa-miR-3689a-5p	3256	4277	discovered in female reproductuve tract and peripheral blood		
hsa-miR-3689b-3p	3257	4278	discovered in female reproductuve tract and peripheral blood		
hsa-miR-3689b-5p	3258	4279	discovered in female reproductuve tract		
hsa-miR-3689c	3259	4280	discovered in B		

			cells		
hsa-miR-3689d	3260	4281	discovered in B cells		
hsa-miR-3689e	3261	4282	discovered in B cells		
hsa-miR-3689f	3262	4283	discovered in B cells		
hsa-miR-3690	3263	4284	discovered in peripheral blood		
hsa-miR-3691-3p	3264	4285	discovered in peripheral blood		
hsa-miR-3691-5p	3265	4286	discovered in peripheral blood		
hsa-miR-3692-3p	3266	4287	discovered in peripheral blood		
hsa-miR-3 692-5p	3267	4288	discovered in peripheral blood		
hsa-miR-369-3p	3268	4289	stem cells		reprogramming
hsa-miR-3 69-5p	3269	4290	stem cells		reprogramming
hsa-miR-3 70	3270	4291		acute meyloid leukaemia and other cancers	tumor suppressor, lipid metabolism
hsa-miR-3713	3271	4292	discovered in neuroblastoma		
hsa-miR-3714	3272	4293	discovered in neuroblastoma		
hsa-miR-3 7la-3p	3273	4294	serum		
hsa-miR-3 7la-5p	3274	4295	serum		
hsa-miR-371b-3p	3275	4296	serum		
hsa-miR-371b-5p	3276	4297	serum		
hsa-miR-3 72	3277	4298	hematopoietic cells, lung, placental (blood)		
hsa-miR-3 73-3p	3278	4299		breast cancer	
hsa-miR-3 73-5p	3279	4300		breast cancer	
hsa-miR-3 74a-3p	3280	4301	muscle (myoblasts)	breast and lung cancer	myogenic differentiation
hsa-miR-3 74a-5p	3281	4302	muscle (myoblasts)	breast and lung cancer	myogenic differentiation
hsa-miR-3 74b-3p	3282	4303	muscle (myoblasts)		myogenic differentiation
hsa-miR-3 74b-5p	3283	4304	muscle (myoblasts)		myogenic differentiation
hsa-miR-3 74c-3p	3284	4305	muscle (myoblasts)		myogenic differentiation
hsa-miR-3 74c-5p	3285	4306	muscle (myoblasts)		myogenic differentiation
hsa-miR-3 75	3286	4307	pancreas (islet)		
hsa-miR-3 76a-2-5p	3287	4308	regulatory miRs for hematopoietic cells (erythroid,platelet, lympho)		
hsa-miR-3 76a-3p	3288	4309	regulatory miRs for hematopoietic cells (erythroid,platelet,		

			lympho)		
hsa-miR-376a-5p	3289	4310	regulatory miRs for hematopoietic cells (erythroid,platelet, lympho)		
hsa-miR-376b-3p	3290	4311	blood	various cancer cells	autophagy
hsa-miR-376b-5p	3291	4312	blood	various cancer cells	autophagy
hsa-miR-376c-3p	3292	4313	trophoblast	various cancer cells	cell proliferatio
hsa-miR-376c-5p	3293	4314	trophoblast	various cancer cells	cell proliferatio
hsa-miR-377-3p	3294	4315	hematopoietic cells		
hsa-miR-377-5p	3295	4316	hematopoietic cells		
hsa-miR-378a-3p	3296	4317	ovary, lipid metabolism		
hsa-miR-378a-5p	3297	4318	ovary, placenta/trophoblast, lipid metabolism		
hsa-miR-378b	3298	4319	lipid metabolism		
hsa-miR-378c	3299	4320	lipid metabolism		
hsa-miR-378d	3300	4321	lipid metabolism		
hsa-miR-378e	3301	4322	lipid metabolism		
hsa-miR-378f	3302	4323	lipid metabolism		
hsa-miR-378g	3303	4324	lipid metabolism		
hsa-miR-378h	3304	4325	lipid metabolism		
hsa-miR-378i	3305	4326	lipid metabolism		
hsa-miR-378j	3306	4327	lipid metabolism		
hsa-miR-379-3p	3307	4328		various cancers (breast, hepatocytes, colon)	
hsa-miR-379-5p	3308	4329		various cancers (breast, hepatocytes, colon)	
hsa-miR-380-3p	3309	4330	brain	neuroblastoma	
hsa-miR-380-5p	3310	4331	brain, embryonic stem cells	neuroblastoma	
hsa-miR-381-3p	3311	4332	chondrogenesis, lung, brain		
hsa-miR-381-5p	3312	4333	chondrogenesis, lung, brain		
hsa-miR-382-3p	3313	4334	renal epithelial cells		
hsa-miR-382-5p	3314	4335	renal epithelial cells		
hsa-miR-383	3315	4336	testes, brain (medulla)		
hsa-miR-384	3316	4337	epithelial cells		
hsa-miR-3907	3317	4338	discovered in female reproductive tract		
hsa-miR-3908	3318	4339	discovered in female reproductive		

			tract		
hsa-miR-3909	3319	4340	discovered in female reproductive tract		
hsa-miR-3910	3320	4341	discovered in female reproductive tract		
hsa-miR-3911	3321	4342	discovered in breast tumor and female reproductive tract		
hsa-miR-3912	3322	4343	discovered in female reproductive tract		
hsa-miR-3913-3p	3323	4344	discovered in breast tumor and female reproductive tract		
hsa-miR-3913-5p	3324	4345	discovered in breast tumor and female reproductive tract		
hsa-miR-3914	3325	4346	discovered in breast tumor and female reproductive tract		
hsa-miR-3915	3326	4347	discovered in female reproductive tract		
hsa-miR-3916	3327	4348	discovered in female reproductive tract		
hsa-miR-3917	3328	4349	discovered in female reproductive tract		
hsa-miR-3918	3329	4350	discovered in female reproductive tract		
hsa-miR-3919	3330	4351	discovered in female reproductive tract		
hsa-miR-3920	3331	4352	discovered in female reproductive tract		
hsa-miR-3921	3332	4353	discovered in female reproductive tract		
hsa-miR-3922-3p	3333	4354	discovered in breast tumor and female reproductive tract		
hsa-miR-3922-5p	3334	4355	discovered in breast tumor and female reproductive tract		
hsa-miR-3923	3335	4356	discovered in female reproductive tract		
hsa-miR-3924	3336	4357	discovered in female reproductive tract		

hsa-miR-3925-3p	3337	4358	discovered in breast tumor and female reproductive tract		
hsa-miR-3925-5p	3338	4359	discovered in breast tumor and female reproductive tract		
hsa-miR-3926	3339	4360	discovered in female reproductive tract		
hsa-miR-3927-3p	3340	4361	discovered in female reproductive tract and psoriasis		
hsa-miR-3927-5p	3341	4362	discovered in female reproductive tract and psoriasis		
hsa-miR-3928	3342	4363	discovered in female reproductive tract		
hsa-miR-3929	3343	4364	discovered in female reproductive tract		
hsa-miR-3934-3p	3344	4365	discovered in abnormal skin (psoriasis)		
hsa-miR-3934-5p	3345	4366	discovered in abnormal skin (psoriasis)		
hsa-miR-3935	3346	4367			
hsa-miR-3936	3347	4368	discovered in breast tumor and lymphoblastic leukaemia		
hsa-miR-3937	3348	4369			
hsa-miR-3938	3349	4370			
hsa-miR-3939	3350	4371			
hsa-miR-3940-3p	3351	4372	discovered in breast tumor		
hsa-miR-3940-5p	3352	4373	discovered in breast tumor		
hsa-miR-3941	3353	4374			
hsa-miR-3942-3p	3354	4375	discovered in breast tumor and lymphoblastic leukaemia		
hsa-miR-3942-5p	3355	4376	discovered in breast tumor and lymphoblastic leukaemia		
hsa-miR-3943	3356	4377			
hsa-miR-3944-3p	3357	4378	discovered in breast tumor		
hsa-miR-3944-5p	3358	4379	discovered in breast tumor		
hsa-miR-3945	3359	4380			
hsa-miR-3960	3360	4381	osteoblast		

hsa-miR-3972	3361	4382	discovered in Acute Myeloid Leukaemia		
hsa-miR-3973	3362	4383	discovered in Acute Myeloid Leukaemia		
hsa-miR-3974	3363	4384	discovered in Acute Myeloid Leukaemia		
hsa-miR-3975	3364	4385	discovered in Acute Myeloid Leukaemia		
hsa-miR-3976	3365	4386	discovered in Acute Myeloid Leukaemia		
hsa-miR-3977	3366	4387	discovered in Acute Myeloid Leukaemia		
hsa-miR-3978	3367	4388	discovered in Acute Myeloid Leukaemia		
hsa-miR-409-3p	3368	4389		gastric cancer	
hsa-miR-409-5p	3369	4390		gastric cancer	
hsa-miR-410	3370	4391	brain	glioma	
hsa-miR-411-3p	3371	4392		Glioblastoma others	
hsa-miR-411-5p	3372	4393		Glioblastoma others	
hsa-miR-412	3373	4394		upregulated in lung cancer	
hsa-miR-421	3374	4395	endothelial cells	gastric cancer, HCC	
hsa-miR-422a	3375	4396	circulating microRNA (in plasma)		
hsa-miR-423-3p	3376	4397	embryonic stem cells		
hsa-miR-423-5p	3377	4398	heart, embryonic stem cells		
hsa-miR-424-3p	3378	4399	endothelial cells	various cancers(e.g B-lieage ALL), cardiac diseases	pro-angiogenic
hsa-miR-424-5p	3379	4400	endothelial cells	various cancers(e.g B-lieage ALL), cardiac diseases	pro-angiogenic
hsa-miR-4251	3380	4401	discovered in embryonic stem cells and neural precusors		
hsa-miR-4252	3381	4402	discovered in embryonic stem cells and neural precusors		
hsa-miR-4253	3382	4403	discovered in embryonic stem cells and neural precusors		
hsa-miR-425-3p	3383	4404	brain	ovarian cancer, brain tumor	
hsa-miR-4254	3384	4405	discovered in		

			embryonic stem cells and neural precusors		
hsa-miR-4255	3385	4406	discovered in embryonic stem cells and neural precusors		
hsa-miR-425-5p	3386	4407	brain	B-lieage ALL, brain tumor	
hsa-miR-4256	3387	4408	discovered in embryonic stem cells and neural precusors		
hsa-miR-4257	3388	4409	discovered in embryonic stem cells and neural precusors		
hsa-miR-4258	3389	4410	discovered in embryonic stem cells and neural precusors		
hsa-miR-4259	3390	441 1	discovered in embryonic stem cells and neural precusors		
hsa-miR-4260	3391	4412	discovered in embryonic stem cells and neural precusors		
hsa-miR-4261	3392	4413	discovered in embryonic stem cells and neural precusors		
hsa-miR-4262	3393	4414	discovered in embryonic stem cells and neural precusors		
hsa-miR-4263	3394	4415	discovered in embryonic stem cells and neural precusors		
hsa-miR-4264	3395	4416	discovered in embryonic stem cells and neural precusors		
hsa-miR-4265	3396	4417	discovered in embryonic stem cells and neural precusors		
hsa-miR-4266	3397	441 8	discovered in embryonic stem cells and neural precusors		
hsa-miR-4267	3398	4419	discovered in embryonic stem		

			cells and neural precusors		
hsa-miR-4268	3399	4420	discovered in embryonic stem cells and neural precusors		
hsa-miR-4269	3400	4421	discovered in embryonic stem cells and neural precusors		
hsa-miR-4270	3401	4422	discovered in embryonic stem cells and neural precusors		
hsa-miR-4271	3402	4423	discovered in embryonic stem cells and neural precusors		
hsa-miR-4272	3403	4424	discovered in embryonic stem cells and neural precusors		
hsa-miR-4273	3404	4425			
hsa-miR-4274	3405	4426	discovered in embryonic stem cells and neural precusors		
hsa-miR-4275	3406	4427	discovered in embryonic stem cells and neural precusors		
hsa-miR-4276	3407	4428	discovered in embryonic stem cells and neural precusors		
hsa-miR-4277	3408	4429	discovered in embryonic stem cells and neural precusors		
hsa-miR-4278	3409	4430	discovered in embryonic stem cells and neural precusors		
hsa-miR-4279	3410	4431	discovered in embryonic stem cells and neural precusors		
hsa-miR-4280	3411	4432	discovered in embryonic stem cells and neural precusors		
hsa-miR-4281	3412	4433	discovered in embryonic stem cells and neural precusors		

hsa-miR-4282	3413	4434	discovered in embryonic stem cells and neural precusors		
hsa-miR-4283	3414	4435	discovered in embryonic stem cells and neural precusors		
hsa-miR-4284	3415	4436	discovered in embryonic stem cells and neural precusors		
hsa-miR-4285	3416	4437	discovered in embryonic stem cells and neural precusors		
hsa-miR-4286	3417	4438	discovered in embryonic stem cells and neural precusors		
hsa-miR-4287	3418	4439	discovered in embryonic stem cells and neural precusors		
hsa-miR-4288	3419	4440	discovered in embryonic stem cells and neural precusors		
hsa-miR-4289	3420	4441	discovered in embryonic stem cells and neural precusors		
hsa-miR-429	3421	4442	Epithelial cells	various cancers (colorectal, endometrial, gastric, ovarian etc)	
hsa-miR-4290	3422	4443	discovered in embryonic stem cells and neural precusors		
hsa-miR-4291	3423	4444	discovered in embryonic stem cells and neural precusors		
hsa-miR-4292	3424	4445	discovered in embryonic stem cells and neural precusors		
hsa-miR-4293	3425	4446	discovered in embryonic stem cells and neural precusors		
hsa-miR-4294	3426	4447	discovered in embryonic stem		

			cells and neural precusors		
hsa-miR-4295	3427	4448	discovered in embryonic stem cells and neural precusors		
hsa-miR-4296	3428	4449	discovered in embryonic stem cells and neural precusors		
hsa-miR-4297	3429	4450	discovered in embryonic stem cells and neural precusors		
hsa-miR-4298	3430	4451	discovered in embryonic stem cells and neural precusors		
hsa-miR-4299	3431	4452	discovered in embryonic stem cells and neural precusors		
hsa-miR-4300	3432	4453	discovered in embryonic stem cells and neural precusors		
hsa-miR-4301	3433	4454	discovered in embryonic stem cells and neural precusors		
hsa-miR-4302	3434	4455	discovered in embryonic stem cells and neural precusors		
hsa-miR-4303	3435	4456	discovered in embryonic stem cells and neural precusors		
hsa-miR-4304	3436	4457	discovered in embryonic stem cells and neural precusors		
hsa-miR-4305	3437	4458	discovered in embryonic stem cells and neural precusors		
hsa-miR-4306	3438	4459	discovered in embryonic stem cells and neural precusors		
hsa-miR-4307	3439	4460	discovered in embryonic stem cells and neural precusors		
hsa-miR-4308	3440	4461	discovered in		

			embryonic stem cells and neural precusors		
hsa-miR-4309	3441	4462	discovered in embryonic stem cells and neural precusors		
hsa-miR-43 10	3442	4463	discovered in embryonic stem cells and neural precusors		
hsa-miR-43 11	3443	4464	discovered in embryonic stem cells and neural precusors		
hsa-miR-43 12	3444	4465	discovered in embryonic stem cells and neural precusors		
hsa-miR-43 13	3445	4466	discovered in embryonic stem cells and neural precusors		
hsa-miR-43 1-3p	3446	4467		Cancers (follicular lymphoma)	
hsa-miR-43 14	3447	4468	discovered in embryonic stem cells and neural precusors		
hsa-miR-43 15	3448	4469	discovered in embryonic stem cells and neural precusors		
hsa-miR-43 1-5p	3449	4470		Cancers (follicular lymphoma)	
hsa-miR-43 16	3450	4471	discovered in embryonic stem cells and neural precusors		
hsa-miR-43 17	345 1	4472	discovered in embryonic stem cells and neural precusors		
hsa-miR-43 18	3452	4473	discovered in embryonic stem cells and neural precusors		
hsa-miR-43 19	3453	4474	discovered in embryonic stem cells and neural precusors		
hsa-miR-4320	3454	4475	discovered in embryonic stem		

			cells and neural precusors		
hsa-miR-4321	3455	4476	discovered in embryonic stem cells and neural precusors		
hsa-miR-4322	3456	4477	discovered in embryonic stem cells and neural precusors		
hsa-miR-4323	3457	4478	discovered in embryonic stem cells and neural precusors		
hsa-miR-432-3p	3458	4479	myoblast		myogenic differentiation
hsa-miR-4324	3459	4480	discovered in embryonic stem cells and neural precusors		
hsa-miR-4325	3460	4481	discovered in embryonic stem cells and neural precusors		
hsa-miR-432-5p	3461	4482	myoblast		myogenic differentiation
hsa-miR-4326	3462	4483	discovered in embryonic stem cells and neural precusors		
hsa-miR-4327	3463	4484	discovered in embryonic stem cells and neural precusors		
hsa-miR-4328	3464	4485	discovered in embryonic stem cells and neural precusors		
hsa-miR-4329	3465	4486	discovered in embryonic stem cells and neural precusors		
hsa-miR-433	3466	4487		various diseases (cancer, Parkinson's, Chondrodysplasia)	
hsa-miR-4330	3467	4488	discovered in embryonic stem cells and neural precusors		
hsa-miR-4417	3468	4489	discovered in B cells		
hsa-miR-4418	3469	4490	discovered in B cells		

hsa-miR-4419a	3470	4491	discovered in B cells		
hsa-miR-4419b	3471	4492	discovered in B cells		
hsa-miR-4420	3472	4493	discovered in B cells		
hsa-miR-4421	3473	4494	discovered in B cells		
hsa-miR-4422	3474	4495	discovered in breast tumor and B cells		
hsa-miR-4423-3p	3475	4496	discovered in breast tumor, B cells and skin(psoriasis)		
hsa-miR-4423-5p	3476	4497	discovered in breast tumor B cells and skin(psoriasis)		
hsa-miR-4424	3477	4498	discovered in B cells		
hsa-miR-4425	3478	4499	discovered in B cells		
hsa-miR-4426	3479	4500	discovered in B cells		
hsa-miR-4427	3480	4501	discovered in B cells		
hsa-miR-4428	3481	4502	discovered in B cells		
hsa-miR-4429	3482	4503	discovered in B cells		
hsa-miR-4430	3483	4504	discovered in B cells		
hsa-miR-443 1	3484	4505	discovered in B cells		
hsa-miR-4432	3485	4506	discovered in B cells		
hsa-miR-4433-3p	3486	4507	discovered in B cells		
hsa-miR-4433-5p	3487	4508	discovered in B cells		
hsa-miR-4434	3488	4509	discovered in B cells		
hsa-miR-443 5	3489	4510	discovered in B cells		
hsa-miR-443 6a	3490	4511	discovered in breast tumor and B cells		
hsa-miR-443 6b-3p	3491	4512	discovered in breast tumor		
hsa-miR-443 6b-5p	3492	4513	discovered in breast tumor		
hsa-miR-443 7	3493	4514	discovered in B cells		
hsa-miR-443 8	3494	4515	discovered in B cells		
hsa-miR-4439	3495	4516	discovered in B cells		
hsa-miR-4440	3496	4517	discovered in B		

			cells		
hsa-miR-4441	3497	4518	discovered in B cells		
hsa-miR-4442	3498	4519	discovered in B cells		
hsa-miR-4443	3499	4520	discovered in B cells		
hsa-miR-4444	3500	4521	discovered in B cells		
hsa-miR-4445-3p	3501	4522	discovered in B cells		
hsa-miR-4445-5p	3502	4523	discovered in B cells		
hsa-miR-4446-3p	3503	4524	discovered in breast tumor and B cells		
hsa-miR-4446-5p	3504	4525	discovered in breast tumor and B cells		
hsa-miR-4447	3505	4526	discovered in B cells		
hsa-miR-4448	3506	4527	discovered in B cells		
hsa-miR-4449	3507	4528	discovered in B cells		
hsa-miR-4450	3508	4529	discovered in B cells		
hsa-miR-4451	3509	4530	discovered in B cells		
hsa-miR-4452	3510	4531	discovered in B cells		
hsa-miR-4453	3511	4532	discovered in B cells		
hsa-miR-4454	3512	4533	discovered in B cells		
hsa-miR-4455	3513	4534	discovered in B cells		
hsa-miR-4456	3514	4535	discovered in B cells		
hsa-miR-4457	3515	4536	discovered in B cells		
hsa-miR-4458	3516	4537	discovered in B cells		
hsa-miR-4459	3517	4538	discovered in B cells		
hsa-miR-4460	3518	4539	discovered in B cells		
hsa-miR-4461	3519	4540	discovered in B cells		
hsa-miR-4462	3520	4541	discovered in B cells		
hsa-miR-4463	3521	4542	discovered in B cells		
hsa-miR-4464	3522	4543	discovered in B cells		
hsa-miR-4465	3523	4544	discovered in B cells		

hsa-miR-4466	3524	4545	discovered in B cells		
hsa-miR-4467	3525	4546	discovered in breast tumor and B cells		
hsa-miR-4468	3526	4547	discovered in B cells		
hsa-miR-4469	3527	4548	discovered in breast tumor and B cells		
hsa-miR-4470	3528	4549	discovered in B cells		
hsa-miR-4471	4550	5571	discovered in breast tumor and B cells		
hsa-miR-4472	4551	5572	discovered in B cells		
hsa-miR-4473	4552	5573	discovered in B cells		
hsa-miR-4474-3p	4553	5574	discovered in breast tumor, lymphoblastic leukaemia and B cells		
hsa-miR-4474-5p	4554	5575	discovered in breast tumor, lymphoblastic leukaemia and B cells		
hsa-miR-4475	4555	5576	discovered in B cells		
hsa-miR-4476	4556	5577	discovered in B cells		
hsa-miR-4477a	4557	5578	discovered in B cells		
hsa-miR-4477b	4558	5579	discovered in B cells		
hsa-miR-4478	4559	5580	discovered in B cells		
hsa-miR-4479	4560	5581	discovered in B cells		
hsa-miR-448	4561	5582	liver(hepatocytes)	HCC	
hsa-miR-4480	4562	5583	discovered in B cells		
hsa-miR-448 1	4563	5584	discovered in B cells		
hsa-miR-4482-3p	4564	5585	discovered in B cells		
hsa-miR-4482-5p	4565	5586	discovered in B cells		
hsa-miR-4483	4566	5587	discovered in B cells		
hsa-miR-4484	4567	5588	discovered in B cells		
hsa-miR-4485	4568	5589	discovered in B cells		
hsa-miR-4486	4569	5590	discovered in B cells		

hsa-miR-4487	4570	5591	discovered in B cells		
hsa-miR-4488	4571	5592	discovered in B cells		
hsa-miR-4489	4572	5593	discovered in breast tumor and B cells		
hsa-miR-4490	4573	5594	discovered in B cells		
hsa-miR-4491	4574	5595	discovered in B cells		
hsa-miR-4492	4575	5596	discovered in B cells		
hsa-miR-4493	4576	5597	discovered in B cells		
hsa-miR-4494	4577	5598	discovered in B cells		
hsa-miR-4495	4578	5599	discovered in B cells		
hsa-miR-4496	4579	5600	discovered in B cells		
hsa-miR-4497	4580	5601	discovered in B cells		
hsa-miR-4498	4581	5602	discovered in B cells		
hsa-miR-4499	4582	5603	discovered in B cells		
hsa-miR-449a	4583	5604	chondrocytes, ciliated epithelial cells	lung, colonic, ovarian cancer	cell cycle progression and proliferation
hsa-miR-449b-3p	4584	5605	ciliated epithelial cells, other tissues	various cancer cells	cell cycle progression and proliferation
hsa-miR-449b-5p	4585	5606	ciliated epithelial cells, other tissues	various cancer cells	cell cycle progression and proliferation
hsa-miR-449c-3p	4586	5607		epithelial ovarian cancer cells	
hsa-miR-449c-5p	4587	5608		epithelial ovarian cancer cells	
hsa-miR-4500	4588	5609	discovered in B cells		
hsa-miR-4501	4589	5610	discovered in B cells		
hsa-miR-4502	4590	5611	discovered in B cells		
hsa-miR-4503	4591	5612	discovered in B cells		
hsa-miR-4504	4592	5613	discovered in B cells		
hsa-miR-4505	4593	5614	discovered in B cells		
hsa-miR-4506	4594	5615	discovered in B cells		
hsa-miR-4507	4595	5616	discovered in B cells		

hsa-miR-4508	4596	5617	discovered in B cells		
hsa-miR-4509	4597	5618	discovered in B cells		
hsa-miR-450a-3p	4598	5619			
hsa-miR-450a-5p	4599	5620			
hsa-miR-450b-3p	4600	5621			
hsa-miR-450b-5p	4601	5622			
hsa-miR-4510	4602	5623	discovered in B cells		
hsa-miR-4511	4603	5624	discovered in B cells		
hsa-miR-4512	4604	5625	discovered in B cells		
hsa-miR-4513	4605	5626	discovered in B cells		
hsa-miR-4514	4606	5627	discovered in B cells		
hsa-miR-4515	4607	5628	discovered in B cells		
hsa-miR-4516	4608	5629	discovered in B cells		
hsa-miR-4517	4609	5630	discovered in B cells		
hsa-miR-4518	4610	5631	discovered in B cells		
hsa-miR-4519	4611	5632	discovered in B cells		
hsa-miR-451a	4612	5633	heart, central nevous system, epithelial cells		
hsa-miR-451b	4613	5634	heart, central nevous system, epithelial cells		
hsa-miR-4520a-3p	4614	5635	discovered in breast tumor and B cells, skin(psoriasis)		
hsa-miR-4520a-5p	4615	5636	discovered in breast tumor and B cells, skin(psoriasis)		
hsa-miR-4520b-3p	4616	5637	discovered in breast tumor		
hsa-miR-4520b-5p	4617	5638	discovered in breast tumor		
hsa-miR-4521	4618	5639	discovered in B cells		
hsa-miR-4522	4619	5640	discovered in B cells		
hsa-miR-4523	4620	5641	discovered in B cells		
hsa-miR-452-3p	4621	5642	myoblast	bladder cancer and others	
hsa-miR-4524a-3p	4622	5643	discovered in breast tumor and B cells, skin(psoriasis)		

hsa-miR-4524a-5p	4623	5644	discovered in breast tumor and B cells, skin(psoriasis)		
hsa-miR-4524b-3p	4624	5645	discovered in breast tumor and B cells, skin(psoriasis)		
hsa-miR-4524b-5p	4625	5646	discovered in breast tumor and B cells, skin(psoriasis)		
hsa-miR-4525	4626	5647	discovered in B cells		
hsa-miR-452-5p	4627	5648	myoblast	bladder cancer and others	
hsa-miR-4526	4628	5649	discovered in breast tumor and B cells		
hsa-miR-4527	4629	5650	discovered in B cells		
hsa-miR-4528	4630	5651	discovered in B cells		
hsa-miR-4529-3p	4631	5652	discovered in breast tumor and B cells		
hsa-miR-4529-5p	4632	5653	discovered in breast tumor and B cells		
hsa-miR-4530	4633	5654	discovered in B cells		
hsa-miR-4531	4634	5655	discovered in B cells		
hsa-miR-4532	4635	5656	discovered in B cells		
hsa-miR-4533	4636	5657	discovered in B cells		
hsa-miR-4534	4637	5658	discovered in B cells		
hsa-miR-4535	4638	5659	discovered in B cells		
hsa-miR-4536-3p	4639	5660	discovered in B cells		
hsa-miR-4536-5p	4640	5661	discovered in B cells		
hsa-miR-4537	4641	5662	discovered in B cells		
hsa-miR-4538	4642	5663	discovered in B cells		
hsa-miR-4539	4643	5664	discovered in B cells		
hsa-miR-4540	4644	5665	discovered in B cells		
hsa-miR-454-3p	4645	5666	embryoid body cells, central nevous system, monocytes		
hsa-miR-454-5p	4646	5667	embryoid body cells, central nevous system, monocytes		

hsa-miR-455-3p	4647	5668		basal cell carcinoma, other cancers	
hsa-miR-455-5p	4648	5669		basal cell carcinoma, other cancers	
hsa-miR-4632-3p	4649	5670	discovred in breast tumor		
hsa-miR-4632-5p	4650	5671	discovered in breast tumor		
hsa-miR-4633-3p	4651	5672	discovered in breast tumor		
hsa-miR-4633-5p	4652	5673	discovered in breast tumor		
hsa-miR-4634	4653	5674	discovered in breast tumor		
hsa-miR-4635	4654	5675	discovered in breast tumor		
hsa-miR-4636	4655	5676	discovered in breast tumor		
hsa-miR-4637	4656	5677	discovered in breast tumor and lymphoblastic leukaemia		
hsa-miR-4638-3p	4657	5678	discovered in breast tumor		
hsa-miR-4638-5p	4658	5679	discovered in breast tumor		
hsa-miR-4639-3p	4659	5680	discovered in breast tumor		
hsa-miR-4639-5p	4660	5681	discovered in breast tumor		
hsa-miR-4640-3p	4661	5682	discovered in breast tumor		
hsa-miR-4640-5p	4662	5683	discovered in breast tumor		
hsa-miR-4641	4663	5684	discovered in breast tumor		
hsa-miR-4642	4664	5685	discovered in breast tumor		
hsa-miR-4643	4665	5686	discovered in breast tumor		
hsa-miR-4644	4666	5687	discovered in breast tumor		
hsa-miR-4645-3p	4667	5688	discovered in breast tumor		
hsa-miR-4645-5p	4668	5689	discovered in breast tumor		
hsa-miR-4646-3p	4669	5690	discovered in breast tumor		
hsa-miR-4646-5p	4670	5691	discovered in breast tumor		
hsa-miR-4647	4671	5692	discovered in breast tumor		
hsa-miR-4648	4672	5693	discovered in breast		

			tumor		
hsa-miR-4649-3p	4673	5694	discovered in breast tumor		
hsa-miR-4649-5p	4674	5695	discovered in breast tumor		
hsa-miR-4650-3p	4675	5696	discovered in breast tumor		
hsa-miR-4650-5p	4676	5697	discovered in breast tumor		
hsa-miR-4651	4677	5698	discovered in breast tumor		
hsa-miR-4652-3p	4678	5699	discovered in breast tumor		
hsa-miR-4652-5p	4679	5700	discovered in breast tumor		
hsa-miR-4653-3p	4680	5701	discovered in breast tumor		
hsa-miR-4653-5p	4681	5702	discovered in breast tumor		
hsa-miR-4654	4682	5703	discovered in breast tumor		
hsa-miR-4655-3p	4683	5704	discovered in breast tumor		
hsa-miR-4655-5p	4684	5705	discovered in breast tumor		
hsa-miR-4656	4685	5706	discovered in breast tumor		
hsa-miR-4657	4686	5707	discovered in breast tumor		
hsa-miR-4658	4687	5708	discovered in breast tumor		
hsa-miR-4659a-3p	4688	5709	discovered in breast tumor		
hsa-miR-4659a-5p	4689	5710	discovered in breast tumor		
hsa-miR-4659b-3p	4690	5711	discovered in breast tumor		
hsa-miR-4659b-5p	4691	5712	discovered in breast tumor		
hsa-miR-466	4692	5713			
hsa-miR-4660	4693	5714	discovered in breast tumor		
hsa-miR-4661-3p	4694	5715	discovered in breast tumor		
hsa-miR-4661-5p	4695	5716	discovered in breast tumor		
hsa-miR-4662a-3p	4696	5717	discovered in breast tumor, psoriasis		
hsa-miR-4662a-5p	4697	5718	discovered in breast tumor, psoriasis		
hsa-miR-4662b	4698	5719	discovered in breast tumor		
hsa-miR-4663	4699	5720	discovered in breast tumor		
hsa-miR-4664-3p	4700	5721	discovered in breast		

			tumor		
hsa-miR-4664-5p	4701	5722	discovered in breast tumor		
hsa-miR-4665-3p	4702	5723	discovered in breast tumor		
hsa-miR-4665-5p	4703	5724	discovered in breast tumor		
hsa-miR-4666a-3p	4704	5725	discovered in breast tumor		
hsa-miR-4666a-5p	4705	5726	discovered in breast tumor		
hsa-miR-4666b	4706	5727			
hsa-miR-4667-3p	4707	5728	discovered in breast tumor		
hsa-miR-4667-5p	4708	5729	discovered in breast tumor		
hsa-miR-4668-3p	4709	5730	discovered in breast tumor		
hsa-miR-4668-5p	4710	5731	discovered in breast tumor		
hsa-miR-4669	4711	5732	discovered in breast tumor		
hsa-miR-4670-3p	4712	5733	discovered in breast tumor		
hsa-miR-4670-5p	4713	5734	discovered in breast tumor		
hsa-miR-4671-3p	4714	5735	discovered in breast tumor		
hsa-miR-4671-5p	4715	5736	discovered in breast tumor		
hsa-miR-4672	4716	5737	discovered in breast tumor		
hsa-miR-4673	4717	5738	discovered in breast tumor		
hsa-miR-4674	4718	5739	discovered in breast tumor		
hsa-miR-4675	4719	5740	discovered in breast tumor		
hsa-miR-4676-3p	4720	5741	discovered in breast tumor		
hsa-miR-4676-5p	4721	5742	discovered in breast tumor		
hsa-miR-4677-3p	4722	5743	discovered in breast tumor, psoriasis		
hsa-miR-4677-5p	4723	5744	discovered in breast tumor, psoriasis		
hsa-miR-4678	4724	5745	discovered in breast tumor		
hsa-miR-4679	4725	5746	discovered in breast tumor		
hsa-miR-4680-3p	4726	5747	discovered in breast tumor		
hsa-miR-4680-5p	4727	5748	discovered in breast tumor		
hsa-miR-4681	4728	5749	discovered in breast		

			tumor		
hsa-miR-4682	4729	5750	discovered in breast tumor		
hsa-miR-4683	4730	5751	discovered in breast tumor		
hsa-miR-4684-3p	4731	5752	discovered in breast tumor		
hsa-miR-4684-5p	4732	5753	discovered in breast tumor		
hsa-miR-4685-3p	4733	5754	discovered in breast tumor		
hsa-miR-4685-5p	4734	5755	discovered in breast tumor		
hsa-miR-4686	4735	5756	discovered in breast tumor		
hsa-miR-4687-3p	4736	5757	discovered in breast tumor		
hsa-miR-4687-5p	4737	5758	discovered in breast tumor		
hsa-miR-4688	4738	5759	discovered in breast tumor		
hsa-miR-4689	4739	5760	discovered in breast tumor		
hsa-miR-4690-3p	4740	5761	discovered in breast tumor		
hsa-miR-4690-5p	4741	5762	discovered in breast tumor		
hsa-miR-4691-3p	4742	5763	discovered in breast tumor		
hsa-miR-4691-5p	4743	5764	discovered in breast tumor		
hsa-miR-4692	4744	5765	discovered in breast tumor		
hsa-miR-4693-3p	4745	5766	discovered in breast tumor		
hsa-miR-4693-5p	4746	5767	discovered in breast tumor		
hsa-miR-4694-3p	4747	5768	discovered in breast tumor		
hsa-miR-4694-5p	4748	5769	discovered in breast tumor		
hsa-miR-4695-3p	4749	5770	discovered in breast tumor		
hsa-miR-4695-5p	4750	5771	discovered in breast tumor		
hsa-miR-4696	4751	5772	discovered in breast tumor		
hsa-miR-4697-3p	4752	5773	discovered in breast tumor		
hsa-miR-4697-5p	4753	5774	discovered in breast tumor		
hsa-miR-4698	4754	5775	discovered in breast tumor		
hsa-miR-4699-3p	4755	5776	discovered in breast tumor		

hsa-miR-4699-5p	4756	5777	discovered in breast tumor		
hsa-miR-4700-3p	4757	5778	discovered in breast tumor		
hsa-miR-4700-5p	4758	5779	discovered in breast tumor		
hsa-miR-4701-3p	4759	5780	discovered in breast tumor		
hsa-miR-4701-5p	4760	5781	discovered in breast tumor		
hsa-miR-4703-3p	4761	5782	discovered in breast tumor		
hsa-miR-4703-5p	4762	5783	discovered in breast tumor		
hsa-miR-4704-3p	4763	5784	discovered in breast tumor		
hsa-miR-4704-5p	4764	5785	discovered in breast tumor		
hsa-miR-4705	4765	5786	discovered in breast tumor		
hsa-miR-4706	4766	5787	discovered in breast tumor		
hsa-miR-4707-3p	4767	5788	discovered in breast tumor		
hsa-miR-4707-5p	4768	5789	discovered in breast tumor		
hsa-miR-4708-3p	4769	5790	discovered in breast tumor		
hsa-miR-4708-5p	4770	5791	discovered in breast tumor		
hsa-miR-4709-3p	4771	5792	discovered in breast tumor		
hsa-miR-4709-5p	4772	5793	discovered in breast tumor		
hsa-miR-4710	4773	5794	discovered in breast tumor		
hsa-miR-4711-3p	4774	5795	discovered in breast tumor		
hsa-miR-4711-5p	4775	5796	discovered in breast tumor		
hsa-miR-4712-3p	4776	5797	discovered in breast tumor		
hsa-miR-4712-5p	4777	5798	discovered in breast tumor		
hsa-miR-4713-3p	4778	5799	discovered in breast tumor		
hsa-miR-4713-5p	4779	5800	discovered in breast tumor		
hsa-miR-4714-3p	4780	5801	discovered in breast tumor		
hsa-miR-4714-5p	4781	5802	discovered in breast tumor		
hsa-miR-4715-3p	4782	5803	discovered in breast tumor		
hsa-miR-4715-5p	4783	5804	discovered in breast		

			tumor		
hsa-miR-4716-3p	4784	5805	discovered in breast tumor		
hsa-miR-4716-5p	4785	5806	discovered in breast tumor		
hsa-miR-4717-3p	4786	5807	discovered in breast tumor		
hsa-miR-4717-5p	4787	5808	discovered in breast tumor		
hsa-miR-4718	4788	5809	discovered in breast tumor		
hsa-miR-4719	4789	5810	discovered in breast tumor		
hsa-miR-4720-3p	4790	5811	discovered in breast tumor		
hsa-miR-4720-5p	4791	5812	discovered in breast tumor		
hsa-miR-4721	4792	5813	discovered in breast tumor		
hsa-miR-4722-3p	4793	5814	discovered in breast tumor		
hsa-miR-4722-5p	4794	5815	discovered in breast tumor		
hsa-miR-4723-3p	4795	5816	discovered in breast tumor		
hsa-miR-4723-5p	4796	5817	discovered in breast tumor		
hsa-miR-4724-3p	4797	5818	discovered in breast tumor		
hsa-miR-4724-5p	4798	5819	discovered in breast tumor		
hsa-miR-4725-3p	4799	5820	discovered in breast tumor		
hsa-miR-4725-5p	4800	5821	discovered in breast tumor		
hsa-miR-4726-3p	4801	5822	discovered in breast tumor		
hsa-miR-4726-5p	4802	5823	discovered in breast tumor		
hsa-miR-4727-3p	4803	5824	discovered in breast tumor		
hsa-miR-4727-5p	4804	5825	discovered in breast tumor		
hsa-miR-4728-3p	4805	5826	discovered in breast tumor		
hsa-miR-4728-5p	4806	5827	discovered in breast tumor		
hsa-miR-4729	4807	5828	discovered in breast tumor		
hsa-miR-4730	4808	5829	discovered in breast tumor		
hsa-miR-4731-3p	4809	5830	discovered in breast tumor		
hsa-miR-4731-5p	4810	5831	discovered in breast tumor		

hsa-miR-4732-3p	4811	5832	discovered in breast tumor		
hsa-miR-4732-5p	4812	5833	discovered in breast tumor		
hsa-miR-4733-3p	4813	5834	discovered in breast tumor		
hsa-miR-4733-5p	4814	5835	discovered in breast tumor		
hsa-miR-4734	4815	5836	discovered in breast tumor		
hsa-miR-4735-3p	4816	5837	discovered in breast tumor		
hsa-miR-4735-5p	4817	5838	discovered in breast tumor		
hsa-miR-4736	4818	5839	discovered in breast tumor		
hsa-miR-4737	4819	5840	discovered in breast tumor		
hsa-miR-4738-3p	4820	5841	discovered in breast tumor		
hsa-miR-4738-5p	4821	5842	discovered in breast tumor		
hsa-miR-4739	4822	5843	discovered in breast tumor		
hsa-miR-4740-3p	4823	5844	discovered in breast tumor		
hsa-miR-4740-5p	4824	5845	discovered in breast tumor		
hsa-miR-4741	4825	5846	discovered in breast tumor, psoriasis		
hsa-miR-4742-3p	4826	5847	discovered in breast tumor, psoriasis		
hsa-miR-4742-5p	4827	5848	discovered in breast tumor		
hsa-miR-4743-3p	4828	5849	discovered in breast tumor		
hsa-miR-4743-5p	4829	5850	discovered in breast tumor		
hsa-miR-4744	4830	5851	discovered in breast tumor		
hsa-miR-4745-3p	4831	5852	discovered in breast tumor		
hsa-miR-4745-5p	4832	5853	discovered in breast tumor		
hsa-miR-4746-3p	4833	5854	discovered in breast tumor		
hsa-miR-4746-5p	4834	5855	discovered in breast tumor		
hsa-miR-4747-3p	4835	5856	discovered in breast tumor		
hsa-miR-4747-5p	4836	5857	discovered in breast tumor		
hsa-miR-4748	4837	5858	discovered in breast tumor		
hsa-miR-4749-3p	4838	5859	discovered in breast		

			tumor		
hsa-miR-4749-5p	4839	5860	discovered in breast tumor		
hsa-miR-4750-3p	4840	5861	discovered in breast tumor		
hsa-miR-4750-5p	4841	5862	discovered in breast tumor		
hsa-miR-4751	4842	5863	discovered in breast tumor		
hsa-miR-4752	4843	5864	discovered in breast tumor		
hsa-miR-4753-3p	4844	5865	discovered in breast tumor		
hsa-miR-4753-5p	4845	5866	discovered in breast tumor		
hsa-miR-4754	4846	5867	discovered in breast tumor		
hsa-miR-4755-3p	4847	5868	discovered in breast tumor		
hsa-miR-4755-5p	4848	5869	discovered in breast tumor		
hsa-miR-4756-3p	4849	5870	discovered in breast tumor		
hsa-miR-4756-5p	4850	5871	discovered in breast tumor		
hsa-miR-4757-3p	4851	5872	discovered in breast tumor		
hsa-miR-4757-5p	4852	5873	discovered in breast tumor		
hsa-miR-4758-3p	4853	5874	discovered in breast tumor		
hsa-miR-4758-5p	4854	5875	discovered in breast tumor		
hsa-miR-4759	4855	5876	discovered in breast tumor		
hsa-miR-4760-3p	4856	5877	discovered in breast tumor		
hsa-miR-4760-5p	4857	5878	discovered in breast tumor		
hsa-miR-4761-3p	4858	5879	discovered in breast tumor		
hsa-miR-4761-5p	4859	5880	discovered in breast tumor		
hsa-miR-4762-3p	4860	5881	discovered in breast tumor		
hsa-miR-4762-5p	4861	5882	discovered in breast tumor		
hsa-miR-4763-3p	4862	5883	discovered in breast tumor		
hsa-miR-4763-5p	4863	5884	discovered in breast tumor		
hsa-miR-4764-3p	4864	5885	discovered in breast tumor		
hsa-miR-4764-5p	4865	5886	discovered in breast tumor		

hsa-miR-4765	4866	5887	discovered in breast tumor		
hsa-miR-4766-3p	4867	5888	discovered in breast tumor		
hsa-miR-4766-5p	4868	5889	discovered in breast tumor		
hsa-miR-4767	4869	5890	discovered in breast tumor		
hsa-miR-4768-3p	4870	5891	discovered in breast tumor		
hsa-miR-4768-5p	4871	5892	discovered in breast tumor		
hsa-miR-4769-3p	4872	5893	discovered in breast tumor		
hsa-miR-4769-5p	4873	5894	discovered in breast tumor		
hsa-miR-4770	4874	5895	discovered in breast tumor		
hsa-miR-4771	4875	5896	discovered in breast tumor		
hsa-miR-4772-3p	4876	5897	discovered in breast tumor, blood monoclear cells	energy metabolism/ obesity	
hsa-miR-4772-5p	4877	5898	discovered in breast tumor, blood monoclear cells	energy metabolism/ obesity	
hsa-miR-4773	4878	5899	discovered in breast tumor		
hsa-miR-4774-3p	4879	5900	discovered in breast tumor and Lymphoblastic leukemia		
hsa-miR-4774-5p	4880	5901	discovered in breast tumor and Lymphoblastic leukemia		
hsa-miR-4775	4881	5902	discovered in breast tumor		
hsa-miR-4776-3p	4882	5903	discovered in breast tumor		
hsa-miR-4776-5p	4883	5904	discovered in breast tumor		
hsa-miR-4777-3p	4884	5905	discovered in breast tumor		
hsa-miR-4777-5p	4885	5906	discovered in breast tumor		
hsa-miR-4778-3p	4886	5907	discovered in breast tumor		
hsa-miR-4778-5p	4887	5908	discovered in breast tumor		
hsa-miR-4779	4888	5909	discovered in breast tumor		
hsa-miR-4780	4889	5910	discovered in breast tumor		
hsa-miR-4781-3p	4890	5911	discovered in breast		

			tumor		
hsa-miR-4781-5p	4891	5912	discovered in breast tumor		
hsa-miR-4782-3p	4892	5913	discovered in breast tumor		
hsa-miR-4782-5p	4893	5914	discovered in breast tumor		
hsa-miR-4783-3p	4894	5915	discovered in breast tumor		
hsa-miR-4783-5p	4895	5916	discovered in breast tumor		
hsa-miR-4784	4896	5917	discovered in breast tumor		
hsa-miR-4785	4897	5918	discovered in breast tumor		
hsa-miR-4786-3p	4898	5919	discovered in breast tumor		
hsa-miR-4786-5p	4899	5920	discovered in breast tumor		
hsa-miR-4787-3p	4900	5921	discovered in breast tumor		
hsa-miR-4787-5p	4901	5922	discovered in breast tumor		
hsa-miR-4788	4902	5923	discovered in breast tumor		
hsa-miR-4789-3p	4903	5924	discovered in breast tumor		
hsa-miR-4789-5p	4904	5925	discovered in breast tumor		
hsa-miR-4790-3p	4905	5926	discovered in breast tumor		
hsa-miR-4790-5p	4906	5927	discovered in breast tumor		
hsa-miR-4791	4907	5928	discovered in breast tumor		
hsa-miR-4792	4908	5929	discovered in breast tumor		
hsa-miR-4793-3p	4909	5930	discovered in breast tumor		
hsa-miR-4793-5p	4910	5931	discovered in breast tumor		
hsa-miR-4794	4911	5932	discovered in breast tumor		
hsa-miR-4795-3p	4912	5933	discovered in breast tumor		
hsa-miR-4795-5p	4913	5934	discovered in breast tumor		
hsa-miR-4796-3p	4914	5935	discovered in breast tumor		
hsa-miR-4796-5p	4915	5936	discovered in breast tumor		
hsa-miR-4797-3p	4916	5937	discovered in breast tumor		
hsa-miR-4797-5p	4917	5938	discovered in breast tumor		

hsa-miR-4798-3p	4918	5939	discovered in breast tumor		
hsa-miR-4798-5p	4919	5940	discovered in breast tumor		
hsa-miR-4799-3p	4920	5941	discovered in breast tumor		
hsa-miR-4799-5p	4921	5942	discovered in breast tumor		
hsa-miR-4800-3p	4922	5943	discovered in breast tumor		
hsa-miR-4800-5p	4923	5944	discovered in breast tumor		
hsa-miR-4801	4924	5945	discovered in breast tumor		
hsa-miR-4802-3p	4925	5946	discovered in breast tumor, psoriasis		
hsa-miR-4802-5p	4926	5947	discovered in breast tumor, psoriasis		
hsa-miR-4803	4927	5948	discovered in breast tumor		
hsa-miR-4804-3p	4928	5949	discovered in breast tumor		
hsa-miR-4804-5p	4929	5950	discovered in breast tumor		
hsa-miR-483-3p	4930	5951		aderonocortical carcinoma, rectal/pancreatic cancer, proliferation of wounded epithelial cells	oncogenic
hsa-miR-483-5p	4931	5952	cartilage (chondrocyte), fetal brain	aderonocortical carcinoma	angiogenesis
hsa-miR-484	4932	5953			mitochondrial network
hsa-miR-485-3p	4933	5954			
hsa-miR-485-5p	4934	5955		ovarian epithelial tumor	
hsa-miR-486-3p	4935	5956	erythroid cells	various cancers	
hsa-miR-486-5p	4936	5957	stem cells (adipose)	various cancers	
hsa-miR-487a	4937	5958		laryngeal carcinoma	
hsa-miR-487b	4938	5959		neuroblastoma,pulmonary carcinogenesis	
hsa-miR-488-3p	4939	5960		prostate cancer, others	
hsa-miR-488-5p	4940	5961		prostate cancer, others	
hsa-miR-489	4941	5962	mesenchymal stem cells	osteogenesis	
hsa-miR-490-3p	4942	5963		neuroblastoma, terine leiomyoma (ULM)/muscle	

hsa-miR-490-5p	4943	5964		neuroblastoma, terine leiomyoma (ULM)/muscle	
hsa-miR-491-3p	4944	5965		various cancers, brain disease	pro-apoptosis
hsa-miR-491-5p	4945	5966		various cancers, brain disease	pro-apoptosis
hsa-miR-492	4946	5967			
hsa-miR-493-3p	4947	5968	myeloid cells, pancreas (islet)		
hsa-miR-493-5p	4948	5969	myeloid cells, pancreas (islet)		
hsa-miR-494	4949	5970	epithelial cells	various cancers	cell cycle
hsa-miR-495-3p	4950	5971	platelet	various cancers (gastric, MLL leukemia, pancreatic etc) and inflammation	
hsa-miR-495-5p	4951	5972	platelet	various cancers (gastric, MLL leukemia, pancreatic etc) and inflammation	
hsa-miR-496	4952	5973	Blood		
hsa-miR-497-3p	4953	5974		various cancers (breast, colorectal, etc)	tumor supressor/pro-apoptosis
hsa-miR-497-5p	4954	5975		various cancers (breast, colorectal, etc)	tumor supressor/pro-apoptosis
hsa-miR-498	4955	5976		autoimmuno (e.g. rheumatoid arthritis)	
hsa-miR-4999-3p	4956	5977			
hsa-miR-4999-5p	4957	5978			
hsa-miR-499a-3p	4958	5979	heart, cardiac stem cells	cardiovascular disease	cardiomyocyte differentiation
hsa-miR-499a-5p	4959	5980	heart, cardiac stem cells	cardiovascular disease	cardiomyocyte differentiation
hsa-miR-499b-3p	4960	5981	heart, cardiac stem cells	cardiovascular disease	cardiomyocyte differentiation
hsa-miR-499b-5p	4961	5982	heart, cardiac stem cells	cardiovascular disease	cardiomyocyte differentiation
hsa-miR-5000-3p	4962	5983	discovered in lymphoblastic leukaemia		
hsa-miR-5000-5p	4963	5984	discovered in lymphoblastic leukaemia		
hsa-miR-5001-3p	4964	5985			
hsa-miR-5001-5p	4965	5986			
hsa-miR-5002-3p	4966	5987			
hsa-miR-5002-5p	4967	5988			
hsa-miR-5003-3p	4968	5989			
hsa-miR-5003-5p	4969	5990			

hsa-miR-5004-3p	4970	5991			
hsa-miR-5004-5p	4971	5992			
hsa-miR-5006-3p	4972	5993	discovered in lymphoblastic leukaemia		
hsa-miR-5006-5p	4973	5994	discovered in lymphoblastic leukaemia		
hsa-miR-5007-3p	4974	5995			
hsa-miR-5007-5p	4975	5996			
hsa-miR-5008-3p	4976	5997			
hsa-miR-5008-5p	4977	5998			
hsa-miR-5009-3p	4978	5999			
hsa-miR-5009-5p	4979	6000			
hsa-miR-500a-3p	4980	6001			
hsa-miR-500a-5p	4981	6002			
hsa-miR-500b	4982	6003	Blood (plasma)		
hsa-miR-5010-3p	4983	6004		abnormal skin (psoriasis)	
hsa-miR-5010-5p	4984	6005		abnormal skin (psoriasis)	
hsa-miR-5011-3p	4985	6006			
hsa-miR-5011-5p	4986	6007			
hsa-miR-501-3p	4987	6008			
hsa-miR-501-5p	4988	6009			
hsa-miR-502-3p	4989	6010		various cancers (hepatocellular, ovarian, breast)	
hsa-miR-502-5p	4990	6011		various cancers (hepatocellular, ovarian, breast)	
hsa-miR-503-3p	4991	6012	ovary		
hsa-miR-503-5p	4992	6013	ovary		
hsa-miR-504	4993	6014		glioblastoma	
hsa-miR-5047	4994	6015			
hsa-miR-505-3p	4995	6016		breast cancer	
hsa-miR-505-5p	4996	6017		breast cancer	
hsa-miR-506-3p	4997	6018		various cancers	
hsa-miR-506-5p	4998	6019		various cancers	
hsa-miR-507	4999	6020			
hsa-miR-508-3p	5000	6021		renal cell carcinoma	
hsa-miR-508-5p	5001	6022	endothelial progenitor cells (EPCs)		
hsa-miR-5087	5002	6023			
hsa-miR-5088	5003	6024			
hsa-miR-5089-3p	5004	6025			
hsa-miR-5089-5p	5005	6026			
hsa-miR-5090	5006	6027			
hsa-miR-5091	5007	6028			
hsa-miR-5092	5008	6029			
hsa-miR-5093	5009	6030			
hsa-miR-509-3-5p	5010	6031	testis		

hsa-miR-509-3p	5011	6032		renal cell carcinoma, brain disease	
hsa-miR-5094	5012	6033			
hsa-miR-5095	5013	6034		cervical cancer	
hsa-miR-509-5p	5014	6035		metabolic syndrome, brain disease	
hsa-miR-5096	5015	6036		cervical cance	
hsa-miR-510	5016	6037	brain		
hsa-miR-5100	5017	6038	discoverd in Salivary gland		
hsa-miR-511	5018	6039	dendritic cells and macrophages		
hsa-miR-512-3p	5019	6040	embryonic stem cells, placenta		
hsa-miR-512-5p	5020	6041	embryonic stem cells, placenta,		
hsa-miR-513a-3p	5021	6042		lung carcinoma	
hsa-miR-513a-5p	5022	6043	endothelial cells		
hsa-miR-513b	5023	6044		follicular lymphoma	
hsa-miR-513c-3p	5024	6045			
hsa-miR-513c-5p	5025	6046			
hsa-miR-514a-3p	5026	6047			
hsa-miR-514a-5p	5027	6048			
hsa-miR-514b-3p	5028	6049		various cancer cells	
hsa-miR-514b-5p	5029	6050		various cancer cells	
hsa-miR-515-3p	5030	6051			
hsa-miR-515-5p	5031	6052	placenta		
hsa-miR-516a-3p	5032	6053	frontal cortex		
hsa-miR-516a-5p	5033	6054	placenta		
hsa-miR-516b-3p	5034	6055			
hsa-miR-516b-5p	5035	6056			
hsa-miR-517-5p	5036	6057	placenta		
hsa-miR-517a-3p	5037	6058	placenta		
hsa-miR-517b-3p	5038	6059	placenta		
hsa-miR-517c-3p	5039	6060	placenta		
hsa-miR-5186	5040	6061	discovered in lymphoblastic leukaemia		
hsa-miR-5187-3p	5041	6062	discovered in lymphoblastic leukaemia, skin (psoriasis)		
hsa-miR-5187-5p	5042	6063	discovered in lymphoblastic leukaemia, skin (psoriasis)		
hsa-miR-5188	5043	6064	discovered in lymphoblastic leukaemia		

hsa-miR-5 189	5044	6065	discovered in lymphoblastic leukaemia		
hsa-miR-5 18a-3p	5045	6066		HCC	
hsa-miR-5 18a-5p	5046	6067		various cancer cells	
hsa-miR-5 18b	5047	6068	placenta	HCC	cell cycle progression
hsa-miR-5 18c-3p	5048	6069	placenta		
hsa-miR-5 18c-5p	5049	6070	placenta		
hsa-miR-5 18d-3p	5050	6071			
hsa-miR-5 18d-5p	5051	6072			
hsa-miR-5 18e-3p	5052	6073		HCC	cell cycle progression
hsa-miR-5 18e-5p	5053	6074		HCC	cell cycle progression
hsa-miR-5 18f-3p	5054	6075	placenta		
hsa-miR-5 18f-5p	5055	6076	placenta		
hsa-miR-5 190	5056	6077	discovered in lymphoblastic leukaemia		
hsa-miR-5 191	5057	6078	discovered in lymphoblastic leukaemia		
hsa-miR-5 192	5058	6079	discovered in lymphoblastic leukaemia		
hsa-miR-5 193	5059	6080	discovered in lymphoblastic leukaemia		
hsa-miR-5 194	5060	6081	discovered in lymphoblastic leukaemia		
hsa-miR-5 195-3p	5061	6082	discovered in lymphoblastic leukaemia		
hsa-miR-5 195-5p	5062	6083	discovered in lymphoblastic leukaemia		
hsa-miR-5 196-3p	5063	6084	discovered in lymphoblastic leukaemia		
hsa-miR-5 196-5p	5064	6085	discovered in lymphoblastic leukaemia		
hsa-miR-5 197-3p	5065	6086	discovered in lymphoblastic leukaemia		
hsa-miR-5 197-5p	5066	6087	discovered in lymphoblastic leukaemia		
hsa-miR-5 19a-3p	5067	6088	placenta	HCC	
hsa-miR-5 19a-5p	5068	6089	placenta	HCC	
hsa-miR-5 19b-3p	5069	6090		breast cancer	
hsa-miR-5 19b-5p	5070	6091		breast cancer	

hsa-miR-519c-3p	5071	6092			
hsa-miR-519c-5p	5072	6093			
hsa-miR-519d	5073	6094	placenta		
hsa-miR-519e-3p	5074	6095	placenta		
hsa-miR-519e-5p	5075	6096	placenta		
hsa-miR-520a-3p	5076	6097	placenta		
hsa-miR-520a-5p	5077	6098	placenta		
hsa-miR-520b	5078	6099		breast cancer	
hsa-miR-520c-3p	5079	6100		gastric cancer, breast tumor	
hsa-miR-520c-5p	5080	6101		breast tumor	
hsa-miR-520d-3p	5081	6102		various cancer cells	
hsa-miR-520d-5p	5082	6103		various cancer cells	
hsa-miR-520e	5083	6104		hepatoma	tomor suppressor
hsa-miR-520f	5084	6105		breast cancer	
hsa-miR-520g	5085	6106		HCC, bladder cancer, breast cancer	
hsa-miR-520h	5086	6107	placental specific		
hsa-miR-521	5087	6108		prostate cancer	
hsa-miR-522-3p	5088	6109		HCC	
hsa-miR-522-5p	5089	6110		HCC	
hsa-miR-523-3p	5090	6111			
hsa-miR-523-5p	5091	6112			
hsa-miR-524-3p	5092	6113		colon cancer stem cells	
hsa-miR-524-5p	5093	6114	placental specific	gliomas	
hsa-miR-525-3p	5094	6115	placental specific	HCC	
hsa-miR-525-5p	5095	6116	placental specific		
hsa-miR-526a	5096	6117	placental specific		
hsa-miR-526b-3p	5097	6118	placental specific		
hsa-miR-526b-5p	5098	6119	placental specific		
hsa-miR-527	5099	6120			
hsa-miR-532-3p	5100	6121		ALL	
hsa-miR-532-5p	5101	6122		ALL	
hsa-miR-539-3p	5102	6123			
hsa-miR-539-5p	5103	6124			
hsa-miR-541-3p	5104	6125			
hsa-miR-541-5p	5105	6126			
hsa-miR-542-3p	5106	6127	monocytes		
hsa-miR-542-5p	5107	6128		basal cell carcinoma, neuroblastoma	
hsa-miR-543	5108	6129			
hsa-miR-544a	5109	6130		osteocarcoma	
hsa-miR-544b	5110	6131		osteocarcoma	
hsa-miR-545-3p	5111	6132			
hsa-miR-545-5p	5112	6133		rectal cancer	
hsa-miR-548	5113	6134			
hsa-miR-548-3p	5114	6135			
hsa-miR-548-5p	5115	6136			

hsa-miR-548a	5116	6137	identified in colorectal microRNAome		
hsa-miR-548a-3p	5117	6138	identified in colorectal microRNAome		
hsa-miR-548a-5p	5118	6139	identified in colorectal microRNAome		
hsa-miR-548aa	5119	6140	identified in cervical tumor		
hsa-miR-548ab	5120	6141	discovered in B-cells		
hsa-miR-548ac	5121	6142	discovered in B-cells		
hsa-miR-548ad	5122	6143	discovered in B-cells		
hsa-miR-548ae	5123	6144	discovered in B-cells		
hsa-miR-548ag	5124	6145	discovered in B-cells		
hsa-miR-548ah-3p	5125	6146	discovered in B-cells		
hsa-miR-548ah-5p	5126	6147	discovered in B-cells		
hsa-miR-548ai	5127	6148	discovered in B-cells		
hsa-miR-548aj-3p	5128	6149	discovered in B-cells		
hsa-miR-548aj-5p	5129	6150	discovered in B-cells		
hsa-miR-548ak	5130	6151	discovered in B-cells		
hsa-miR-548al	5131	6152	discovered in B-cells		
hsa-miR-548am-3p	5132	6153	discovered in B-cells		
hsa-miR-548am-5p	5133	6154	discovered in B-cells		
hsa-miR-548an	5134	6155	discovered in B-cells		
hsa-miR-548ao-3p	5135	6156			
hsa-miR-548ao-5p	5136	6157			
hsa-miR-548ap-3p	5137	6158			
hsa-miR-548ap-5p	5138	6159			
hsa-miR-548aq-3p	5139	6160			
hsa-miR-548aq-5p	5140	6161			
hsa-miR-548ar-3p	5141	6162			
hsa-miR-548ar-5p	5142	6163			
hsa-miR-548as-3p	5143	6164			
hsa-miR-548as-5p	5144	6165			
hsa-miR-548at-3p	5145	6166		prostate cancer	
hsa-miR-548at-5p	5146	6167		prostate cancer	
hsa-miR-548au-3p	5147	6168			

hsa-miR-548au-5p	5148	6169			
hsa-miR-548av-3p	5149	6170			
hsa-miR-548av-5p	5150	6171			
hsa-miR-548aw	5151	6172		prostate cancer	
hsa-miR-548ay-3p	5152	6173	discovered in abnormal skin (psoriasis)		
hsa-miR-548ay-5p	5153	6174	discovered in abnormal skin (psoriasis)		
hsa-miR-548az-3p	5154	6175	discovered in abnormal skin (psoriasis)		
hsa-miR-548az-5p	5155	6176	discovered in abnormal skin (psoriasis)		
hsa-miR-548b-3p	5156	6177	identified in colorectal microRNAome		
hsa-miR-548b-5p	5157	6178	immune cells, frontal cortex		
hsa-miR-548c-3p	5158	6179	identified in colorectal microRNAome		
hsa-miR-548c-5p	5159	6180	immune cells, frontal cortex		
hsa-miR-548d-3p	5160	6181	identified in colorectal microRNAome		
hsa-miR-548d-5p	5161	6182	identified in colorectal microRNAome		
hsa-miR-548e	5162	6183	embryonic stem cells		
hsa-miR-548f	5163	6184	embryonic stem cells		
hsa-miR-548g-3p	5164	6185	embryonic stem cells		
hsa-miR-548g-5p	5165	6186	embryonic stem cells		
hsa-miR-548h-3p	5166	6187	embryonic stem cells		
hsa-miR-548h-5p	5167	6188	embryonic stem cells		
hsa-miR-548i	5168	6189	embryonic stem cells, immune cells		
hsa-miR-548j	5169	6190	immune cells		
hsa-miR-548k	5170	6191	embryonic stem cells		
hsa-miR-5481	5171	6192	embryonic stem cells		
hsa-miR-548m	5172	6193	embryonic stem cells		
hsa-miR-548n	5173	6194	embryonic stem cells, immune cells		

hsa-miR-548o-3p	5174	6195	embryonic stem cells		
hsa-miR-548o-5p	5175	6196	embryonic stem cells		
hsa-miR-548p	5176	6197	embryonic stem cells		
hsa-miR-548q	5177	6198		ovarian cancer cells	
hsa-miR-548s	5178	6199	discovered in the melanoma MicroRNAome		
hsa-miR-548t-3p	5179	6200	discovered in the melanoma MicroRNAome		
hsa-miR-548t-5p	5180	6201	discovered in the melanoma MicroRNAome		
hsa-miR-548u	5181	6202	discovered in the melanoma MicroRNAome		
hsa-miR-548w	5182	6203	discovered in the melanoma MicroRNAome		
hsa-miR-548y	5183	6204			
hsa-miR-548z	5184	6205	discovered in cervical tumor		
hsa-miR-549a	5185	6206	discovered in a colorectal MicroRNAome		
hsa-miR-550a-3-5p	5186	6207		Hepatocellular Carcinoma	
hsa-miR-550a-3p	5187	6208		Hepatocellular Carcinoma	
hsa-miR-550a-5p	5188	6209		Hepatocellular Carcinoma	
hsa-miR-550b-2-5p	5189	6210	discovered in cervical tumor		
hsa-miR-550b-3p	5190	6211	discovered in cervical tumor		
hsa-miR-551a	5191	6212		gastric cancer	
hsa-miR-551b-3p	5192	6213	hepatocytes		
hsa-miR-551b-5p	5193	6214	hepatocytes		
hsa-miR-552	5194	6215	discovered in a colorectal MicroRNAome		
hsa-miR-553	5195	6216	discovered in a colorectal MicroRNAome		
hsa-miR-554	5196	6217	discovered in a colorectal MicroRNAome		
hsa-miR-555	5197	6218	discovered in a colorectal MicroRNAome		
hsa-miR-556-3p	5198	6219	discovered in a		

			colorectal MicroRNAome		
hsa-miR-556-5p	5199	6220	discovered in a colorectal MicroRNAome		
hsa-miR-557	5200	6221	liver(hepatocytes)		
hsa-miR-5571-3p	5201	6222	discoveredd in Salivary gland		
hsa-miR-5571-5p	5202	6223	discoveredd in Salivary gland		
hsa-miR-5572	5203	6224	discoveredd in Salivary gland		
hsa-miR-5579-3p	5204	6225			
hsa-miR-5579-5p	5205	6226			
hsa-miR-558	5206	6227		neuroblastoma	
hsa-miR-5580-3p	5207	6228			
hsa-miR-5580-5p	5208	6229			
hsa-miR-5581-3p	5209	6230			
hsa-miR-5581-5p	5210	6231			
hsa-miR-5582-3p	5211	6232			
hsa-miR-5582-5p	5212	6233			
hsa-miR-5583-3p	5213	6234			
hsa-miR-5583-5p	5214	6235			
hsa-miR-5584-3p	5215	6236			
hsa-miR-5584-5p	5216	6237			
hsa-miR-5585-3p	5217	6238			
hsa-miR-5585-5p	5218	6239			
hsa-miR-5586-3p	5219	6240			
hsa-miR-5586-5p	5220	6241			
hsa-miR-5587-3p	5221	6242			
hsa-miR-5587-5p	5222	6243			
hsa-miR-5588-3p	5223	6244			
hsa-miR-5588-5p	5224	6245			
hsa-miR-5589-3p	5225	6246			
hsa-miR-5589-5p	5226	6247			
hsa-miR-559	5227	6248			
hsa-miR-5590-3p	5228	6249			
hsa-miR-5590-5p	5229	6250			
hsa-miR-5591-3p	5230	6251			
hsa-miR-5591-5p	5231	6252			
hsa-miR-561-3p	5232	6253		multiple myeloma	
hsa-miR-561-5p	5233	6254		multiple myeloma	
hsa-miR-562	5234	6255			
hsa-miR-563	5235	6256	discovered in a colorectal MicroRNAome		
hsa-miR-564	5236	6257		Chronic myeloid leukemia	
hsa-miR-566	5237	6258		MALT lymphoma/lymphocyte	
hsa-miR-567	5238	6259		colorectal cancer	
hsa-miR-568	5239	6260	discovered in a colorectal		

			MicroRNAome		
hsa-miR-5680	5240	6261		Associated with metastatic prostate cancer	
hsa-miR-5681a	5241	6262		Associated with metastatic prostate cancer	
hsa-miR-5681b	5242	6263		Associated with metastatic prostate cancer	
hsa-miR-5682	5243	6264		Associated with metastatic prostate cancer	
hsa-miR-5683	5244	6265		Associated with metastatic prostate cancer	
hsa-miR-5684	5245	6266		Associated with metastatic prostate cancer	
hsa-miR-5685	5246	6267		Associated with metastatic prostate cancer	
hsa-miR-5686	5247	6268		Associated with metastatic prostate cancer	
hsa-miR-5687	5248	6269		Associated with metastatic prostate cancer	
hsa-miR-5688	5249	6270		Associated with metastatic prostate cancer	
hsa-miR-5689	5250	6271		Associated with metastatic prostate cancer	
hsa-miR-569	5251	6272			
hsa-miR-5690	5252	6273		Associated with metastatic prostate cancer	
hsa-miR-5691	5253	6274		Associated with metastatic prostate cancer	
hsa-miR-5692a	5254	6275		Associated with metastatic prostate cancer	
hsa-miR-5692b	5255	6276		Associated with metastatic prostate cancer	
hsa-miR-5692c	5256	6277		Associated with metastatic prostate cancer	
hsa-miR-5693	5257	6278		Associated with metastatic prostate cancer	
hsa-miR-5694	5258	6279		Associated with metastatic	

				prostate cancer	
hsa-miR-5695	5259	6280		Associated with metastatic prostate cancer	
hsa-miR-5696	5260	6281		Associated with metastatic prostate cancer	
hsa-miR-5697	5261	6282		Associated with metastatic prostate cancer	
hsa-miR-5698	5262	6283		Associated with metastatic prostate cancer	
hsa-miR-5699	5263	6284		Associated with metastatic prostate cancer	
hsa-miR-5700	5264	6285		Associated with metastatic prostate cancer	
hsa-miR-5701	5265	6286		Associated with metastatic prostate cancer	
hsa-miR-5702	5266	6287		Associated with metastatic prostate cancer	
hsa-miR-5703	5267	6288		Associated with metastatic prostate cancer	
hsa-miR-570-3p	5268	6289		follicular lymphoma	
hsa-miR-5704	5269	6290		Associated with metastatic prostate cancer	
hsa-miR-5705	5270	6291		Associated with metastatic prostate cancer	
hsa-miR-570-5p	5271	6292		follicular lymphoma	
hsa-miR-5706	5272	6293		Associated with metastatic prostate cancer	
hsa-miR-5707	5273	6294		Associated with metastatic prostate cancer	
hsa-miR-5708	5274	6295		Associated with metastatic prostate cancer	
hsa-miR-571	5275	6296	frontal cortex		
hsa-miR-572	5276	6297	circulating microRNA (in plasma)	basal cell carcinoma	
hsa-miR-573	5277	6298	discovered in the colorectal MicroRNAome		
hsa-miR-5739	5278	6299	endothelial cells		

hsa-miR-574-3p	5279	6300	blood (myeloid cells)	follicular lymphoma	
hsa-miR-574-5p	5280	6301	semen		
hsa-miR-575	5281	6302		gastric cancer	
hsa-miR-576-3p	5282	6303	discovered in a colorectal MicroRNAome		
hsa-miR-576-5p	5283	6304	cartilage/ chondrocyte		
hsa-miR-577	5284	6305	discovered in a colorectal MicroRNAome		
hsa-miR-578	5285	6306	discovered in a colorectal MicroRNAome		
hsa-miR-5787	5286	6307	fibroblast		
hsa-miR-579	5287	6308			
hsa-miR-580	5288	6309		breast cancer	
hsa-miR-581	5289	6310	liver(hepatocytes)		
hsa-miR-582-3p	5290	6311	cartilage/chondrocyte	bladder cancer	
hsa-miR-582-5p	5291	6312		bladder cancer	
hsa-miR-583	5292	6313		rectal cancer cells	
hsa-miR-584-3p	5293	6314		tumor cells (follicular lymphoma, rectal cancer cells)	
hsa-miR-584-5p	5294	6315		tumor cells (follicular lymphoma, rectal cancer cells)	
hsa-miR-585	5295	6316		oral squamous cell carcinoma	
hsa-miR-586	5296	6317	discovered in a colorectal MicroRNAome		
hsa-miR-587	5297	6318	discovered in a colorectal MicroRNAome		
hsa-miR-588	5298	6319	discovered in a colorectal MicroRNAome		
hsa-miR-589-3p	5299	6320	mesothelial cells		
hsa-miR-589-5p	5300	6321	mesothelial cells		
hsa-miR-590-3p	5301	6322	cardiomyocytes		Cell cycle progression
hsa-miR-590-5p	5302	6323	cardiomyocytes		Cell cycle progression
hsa-miR-591	5303	6324		neuroblastoma	
hsa-miR-592	5304	6325		hepatocellular carcinoma	
hsa-miR-593-3p	5305	6326		esophageal cancer	
hsa-miR-593-5p	5306	6327		esophageal cancer	
hsa-miR-595	5307	6328		heart failure	
hsa-miR-596	5308	6329		ependymoma,	

				cancers	
hsa-miR-597	5309	6330	discovered in a colorectal MicroRNAome		
hsa-miR-598	5310	6331	Blood (lymphocytes)		
hsa-miR-599	5311	6332		Multiple sclerosis	
hsa-miR-600	5312	6333	discovered in a colorectal MicroRNAome		
hsa-miR-601	5313	6334		various cancers (colonrectal, gastric)	
hsa-miR-602	5314	6335	oocyte		
hsa-miR-603	5315	6336			
hsa-miR-604	5316	6337	discovered in a colorectal MicroRNAome		
hsa-miR-605	5317	6338	discovered in a colorectal MicroRNAome		
hsa-miR-606	5318	6339	discovered in a colorectal MicroRNAome		
hsa-miR-6068	5319	6340	discovered in endothelial cells		
hsa-miR-6069	5320	6341	discovered in endothelial cells		
hsa-miR-607	5321	6342	discovered in a colorectal MicroRNAome		
hsa-miR-6070	5322	6343	discovered in a colorectal MicroRNAome		
hsa-miR-6071	5323	6344	discovered in endothelial cells		
hsa-miR-6072	5324	6345	discovered in endothelial cells		
hsa-miR-6073	5325	6346	discovered in endothelial cells		
hsa-miR-6074	5326	6347	discovered in endothelial cells		
hsa-miR-6075	5327	6348	discovered in endothelial cells		
hsa-miR-6076	5328	6349	discovered in endothelial cells		
hsa-miR-6077	5329	6350	discovered in endothelial cells		
hsa-miR-6078	5330	6351	discovered in endothelial cells		
hsa-miR-6079	5331	6352	discovered in endothelial cells		
hsa-miR-608	5332	6353		various cancers	
hsa-miR-6080	5333	6354	discovered in endothelial cells		

hsa-miR-6081	5334	6355	discovered in endothelial cells		
hsa-miR-6082	5335	6356	discovered in endothelial cells		
hsa-miR-6083	5336	6357	discovered in endothelial cells		
hsa-miR-6084	5337	6358	discovered in endothelial cells		
hsa-miR-6085	5338	6359	discovered in endothelial cells		
hsa-miR-6086	5339	6360	embryonic stem cells		
hsa-miR-6087	5340	6361	embryonic stem cells		
hsa-miR-6088	5341	6362	embryonic stem cells		
hsa-miR-6089	5342	6363	embryonic stem cells		
hsa-miR-609	5343	6364	discovered in a colorectal MicroRNAome		
hsa-miR-6090	5344	6365	embryonic stem cells		
hsa-miR-610	5345	6366		gastric cancer	
hsa-miR-611	5346	6367		Renal cell carcinoma	
hsa-miR-612	5347	6368		AM leukemia	
hsa-miR-6124	5348	6369			
hsa-miR-6125	5349	6370			
hsa-miR-6126	5350	6371			
hsa-miR-6127	5351	6372			
hsa-miR-6128	5352	6373			
hsa-miR-6129	5353	6374			
hsa-miR-613	5354	6375	lipid metabollism		
hsa-miR-6130	5355	6376			
hsa-miR-6131	5356	6377			
hsa-miR-6132	5357	6378			
hsa-miR-6133	5358	6379			
hsa-miR-6134	5359	6380			
hsa-miR-614	5360	6381	circulating micrRNAs (in Plasma)		
hsa-miR-615-3p	5361	6382			
hsa-miR-615-5p	5362	6383			
hsa-miR-616-3p	5363	6384		prostate cancer	
hsa-miR-6165	5364	6385			Pro-apoptotic factor
hsa-miR-616-5p	5365	6386		prostate cancer	
hsa-miR-617	5366	6387			
hsa-miR-618	5367	6388			
hsa-miR-619	5368	6389	discovered in a colorectal MicroRNAome		
hsa-miR-620	5369	6390	discovered in a		

			colorectal MicroRNAome		
hsa-miR-621	5370	6391			
hsa-miR-622	5371	6392			
hsa-miR-623	5372	6393			
hsa-miR-624-3p	5373	6394	chondrocyte		
hsa-miR-624-5p	5374	6395	chondrocyte		
hsa-miR-625-3p	5375	6396	liver(hepatocytes),circulating (blood)	various cancers	
hsa-miR-625-5p	5376	6397	liver(hepatocytes),circulating (blood)	various cancers	
hsa-miR-626	5377	6398	discovered in the colorectal MicroRNAome		
hsa-miR-627	5378	6399		colorectal cancer	
hsa-miR-628-3p	5379	6400		neuroblastoma	
hsa-miR-628-5p	5380	6401		neuroblastoma	
hsa-miR-629-3p	5381	6402		B-lineage ALL, T cell lupus, RCC/kidney	
hsa-miR-629-5p	5382	6403		B-lineage ALL, T cell lupus, RCC/kidney	
hsa-miR-630	5383	6404	chondrocytes	rectal cancer	
hsa-miR-631	5384	6405	discovered in the colorectal MicroRNAom		
hsa-miR-632	5385	6406		myelodysplastic syndromes	
hsa-miR-633	5386	6407		multiple sclerosis	
hsa-miR-634	5387	6408	cartilage/ chondrocyte		
hsa-miR-635	5388	6409	discovered in the colorectal MicroRNAome		
hsa-miR-636	5389	6410		myelodysplastic syndromes	
hsa-miR-637	5390	6411	discovered in the colorectal MicroRNAome		
hsa-miR-638	5391	6412		Lupus nephritis, basal cell carcinoma	
hsa-miR-639	5392	6413	discovered in the colorectal MicroRNAome		
hsa-miR-640	5393	6414		Chronic lymphocytic leukemia	
hsa-miR-641	5394	6415	cartilage/ chondrocyte		
hsa-miR-642a-3p	5395	6416	adipocyte		
hsa-miR-642a-5p	5396	6417	discovered in the colorectal MicroRNAome		

hsa-miR-642b-3p	5397	6418	discovered in a cervial tumo		
hsa-miR-642b-5p	5398	6419	discovered in a cervial tumo		
hsa-miR-643	5399	6420	discovered in the colorectal MicroRNAome		
hsa-miR-644a	5400	6421			
hsa-miR-645	5401	6422		ovarian cancer	
hsa-miR-646	5402	6423			
hsa-miR-647	5403	6424		prostate and lung cancer	
hsa-miR-648	5404	6425	circulating micrRNAs (in Plasma)		
hsa-miR-649	5405	6426	Serum		
hsa-miR-6499-3p	5406	6427	discovered in abnormal skin (psoriasis)		
hsa-miR-6499-5p	5407	6428	discovered in abnormal skin (psoriasis)		
hsa-miR-650	5408	6429		melanoma	
hsa-miR-6500-3p	5409	6430	discovered in abnormal skin (psoriasis)		
hsa-miR-6500-5p	5410	6431	discovered in abnormal skin (psoriasis)		
hsa-miR-6501-3p	5411	6432	discovered in abnormal skin (psoriasis)		
hsa-miR-6501-5p	5412	6433	discovered in abnormal skin (psoriasis)		
hsa-miR-6502-3p	5413	6434	discovered in abnormal skin (psoriasis)		
hsa-miR-6502-5p	5414	6435	discovered in abnormal skin (psoriasis)		
hsa-miR-6503-3p	5415	6436	discovered in abnormal skin (psoriasis)		
hsa-miR-6503-5p	5416	6437	discovered in abnormal skin (psoriasis)		
hsa-miR-6504-3p	5417	6438	discovered in abnormal skin (psoriasis)		
hsa-miR-6504-5p	5418	6439	discovered in abnormal skin (psoriasis)		
hsa-miR-6505-3p	5419	6440	discovered in abnormal skin		

			(psoriasis)		
hsa-miR-6505-5p	5420	6441	discovered in abnormal skin (psoriasis)		
hsa-miR-6506-3p	5421	6442	discovered in abnormal skin (psoriasis)		
hsa-miR-6506-5p	5422	6443	discovered in abnormal skin (psoriasis)		
hsa-miR-6507-3p	5423	6444	discovered in abnormal skin (psoriasis)		
hsa-miR-6507-5p	5424	6445	discovered in abnormal skin (psoriasis)		
hsa-miR-6508-3p	5425	6446	discovered in abnormal skin (psoriasis)		
hsa-miR-6508-5p	5426	6447	discovered in abnormal skin (psoriasis)		
hsa-miR-6509-3p	5427	6448	discovered in abnormal skin (psoriasis)		
hsa-miR-6509-5p	5428	6449	discovered in abnormal skin (psoriasis)		
hsa-miR-65 1	5429	6450	discovered in the colorectal MicroRNAome	lung cancer	
hsa-miR-65 10-3p	5430	645 1	discovered in abnormal skin (psoriasis)		
hsa-miR-65 10-5p	543 1	6452	discovered in abnormal skin (psoriasis)		
hsa-miR-65 l la-3p	5432	6453	discovered in abnormal skin (psoriasis) and epididymis		
hsa-miR-65 l la-5p	5433	6454	discovered in abnormal skin (psoriasis) and epididymis		
hsa-miR-65 1lb-3p	5434	6455	discovered in epididymis		
hsa-miR-65 1lb-5p	5435	6456	discovered in epididymis		
hsa-miR-65 12-3p	5436	6457	discovered in abnormal skin (psoriasis)		
hsa-miR-65 12-5p	5437	6458	discovered in abnormal skin (psoriasis)		

hsa-miR-6513-3p	5438	6459	discovered in abnormal skin (psoriasis)		
hsa-miR-6513-5p	5439	6460	discovered in abnormal skin (psoriasis)		
hsa-miR-6514-3p	5440	6461	discovered in abnormal skin (psoriasis)		
hsa-miR-6514-5p	5441	6462	discovered in abnormal skin (psoriasis)		
hsa-miR-6515-3p	5442	6463	discovered in abnormal skin (psoriasis) and epididymis		
hsa-miR-6515-5p	5443	6464	discovered in abnormal skin (psoriasis) and epididymis		
hsa-miR-652-3p	5444	6465		rectal cancer cells	
hsa-miR-652-5p	5445	6466		rectal cancer cells	
hsa-miR-653	5446	6467	Discovered in the colorectal MicroRNAome		
hsa-miR-654-3p	5447	6468	Discovered in the colorectal MicroRNAome		
hsa-miR-654-5p	5448	6469	bone marrow	prostate cancer	
hsa-miR-655	5449	6470			
hsa-miR-656	5450	6471		various cancers	
hsa-miR-657	5451	6472	oligodendrocytes	diabetes	
hsa-miR-658	5452	6473		gastric cancer	
hsa-miR-659-3p	5453	6474	myoblast		
hsa-miR-659-5p	5454	6475	myoblast		
hsa-miR-660-3p	5455	6476	myoblast		
hsa-miR-660-5p	5456	6477	myoblast		
hsa-miR-661	5457	6478		breast cancer	
hsa-miR-662	5458	6479	endothelial progenitor cells, oocytes		
hsa-miR-663a	5459	6480		follicular lymphoma, Lupus nephritis	
hsa-miR-663b	5460	6481		follicular lymphoma, Lupus nephritis	
hsa-miR-664a-3p	5461	6482	embryonic stem cells		component of SnoRNAs
hsa-miR-664a-5p	5462	6483	embryonic stem cells		component of SnoRNAs
hsa-miR-664b-3p	5463	6484	embryonic stem cells		component of SnoRNAs
hsa-miR-664b-5p	5464	6485	embryonic stem cells		component of SnoRNAs

hsa-miR-665	5465	6486		breast cancer	
hsa-miR-668	5466	6487	keratinocytes		senescence
hsa-miR-670	5467	6488			
hsa-miR-671-3p	5468	6489			
hsa-miR-6715a-3p	5469	6490	discovered in epididymis		
hsa-miR-6715b-3p	5470	6491	discovered in epididymis		
hsa-miR-6715b-5p	5471	6492	discovered in epididymis		
hsa-miR-671-5p	5472	6493		rectal cancer, prolactinomas	
hsa-miR-6716-3p	5473	6494	discovered in epididymis		
hsa-miR-6716-5p	5474	6495	discovered in epididymis		
hsa-miR-6717-5p	5475	6496	discovered in epididymis		
hsa-miR-6718-5p	5476	6497	discovered in epididymis		
hsa-miR-6719-3p	5477	6498	discovered in epididymis		
hsa-miR-6720-3p	5478	6499	discovered in epididymis		
hsa-miR-6721-5p	5479	6500	discovered in epididymis		
hsa-miR-6722-3p	5480	6501	discovered in epididymis		
hsa-miR-6722-5p	5481	6502	discovered in epididymis		
hsa-miR-6723-5p	5482	6503	discovered in epididymis		
hsa-miR-6724-5p	5483	6504	discovered in epididymis		
hsa-miR-675-3p	5484	6505		adrenocortical tumor	
hsa-miR-675-5p	5485	6506		adrenocortical tumor	
hsa-miR-676-3p	5486	6507	discovered in female reproductuve tract		
hsa-miR-676-5p	5487	6508	discovered in female reproductuve tract		
hsa-miR-708-3p	5488	6509		Various cancers (lung, bladder, pancreatic , ALL)	
hsa-miR-708-5p	5489	6510		Various cancers (lung, bladder, pancreatic , ALL)	
hsa-miR-711	5490	6511		cutaneous T-cell lymphomas	
hsa-miR-7-1-3p	5491	6512	Glioblast, brain, prancreas		
hsa-miR-718	5492	6513	blood		

hsa-miR-7-2-3p	5493	6514	brain, pancreas		
hsa-miR-744-3p	5494	6515	heart		
hsa-miR-744-5p	5495	6516	embryonic stem cells, heart		
hsa-miR-758-3p	5496	6517	cholesterol regulation and brain		
hsa-miR-758-5p	5497	6518	cholesterol regulation and brain		
hsa-miR-759	5498	6519			
hsa-miR-7-5p	5499	6520	brain		
hsa-miR-760	5500	6521		colonrectal and breast cancer	
hsa-miR-761	5501	6522			
hsa-miR-762	5502	6523	corneal epithelial cells		
hsa-miR-764	5503	6524	osteoblast		
hsa-miR-765	5504	6525		rectal cancer	
hsa-miR-766-3p	5505	6526	embryonic stem cells		
hsa-miR-766-5p	5506	6527	embryonic stem cells		
hsa-miR-767-3p	5507	6528	/		
hsa-miR-767-5p	5508	6529	/		
hsa-miR-769-3p	5509	6530			
hsa-miR-769-5p	5510	6531			
hsa-miR-770-5p	5511	6532			
hsa-miR-802	5512	6533	brain , epithelial cells, hepatocytes	down symdrome	
hsa-miR-873-3p	5513	6534			
hsa-miR-873-5p	5514	6535			
hsa-miR-874	5515	6536		cervical cancer, lung cancer, carcinoma	
hsa-miR-875-3p	5516	6537			
hsa-miR-875-5p	5517	6538			
hsa-miR-876-3p	5518	6539			
hsa-miR-876-5p	5519	6540			
hsa-miR-877-3p	5520	6541			
hsa-miR-877-5p	5521	6542			
hsa-miR-885-3p	5522	6543	embryonic stem cells		
hsa-miR-885-5p	5523	6544	embryonic stem cells		
hsa-miR-887	5524	6545			
hsa-miR-888-3p	5525	6546			
hsa-miR-888-5p	5526	6547			
hsa-miR-889	5527	6548			
hsa-miR-890	5528	6549	epididymis		
hsa-miR-891a	5529	6550	epididymis	osteosarcoma	
hsa-miR-891b	5530	6551	epididymis		
hsa-miR-892a	5531	6552	epididymis		
hsa-miR-892b	5532	6553	epididymis		
hsa-miR-892c-3p	5533	6554	discovered in epididymis		

hsa-miR-892c-5p	5534	6555	discovered in epididymis		
hsa-miR-920	5535	6556	human testis		
hsa-miR-921	5536	6557	human testis	muscle invasive bladder cancer	
hsa-miR-922	5537	6558	human testis, neuronal tissues	multiple sclerosis, Alcoholic liver disease	
hsa-miR-924	5538	6559	human testis		
hsa-miR-92a-1-5p	5539	6560	endothelial cells		
hsa-miR-92a-2-5p	5540	6561	endothelial cells		
hsa-miR-92a-3p	5541	6562	endothelial cells, CNS		
hsa-miR-92b-3p	5542	6563	endothelial cells, heart		
hsa-miR-92b-5p	5543	6564	endothelial cells, heart		
hsa-miR-933	5544	6565	discovered in cervical cancer		
hsa-miR-93-3p	5545	6566	embryonic stem cells	basal cell carcinoma	
hsa-miR-934	5546	6567	discovered in cervical cancer		
hsa-miR-935	5547	6568	blood monoclear cells	energy metabolism/ obesity, medullablastoma/ neural stem cells	
hsa-miR-93-5p	5548	6569	embryonic stem cells		
hsa-miR-936	5549	6570	skin		
hsa-miR-937-3p	5550	6571		cervical cancer	
hsa-miR-937-5p	5551	6572		cervical cancer	
hsa-miR-938	5552	6573		Various cancer cells	
hsa-miR-939-3p	5553	6574	hepatocytes		
hsa-miR-939-5p	5554	6575	hepatocytes		
hsa-miR-9-3p	5555	6576	brain	Cancers and brain diseases	
hsa-miR-940	5556	6577	identified in Cervical cancer		
hsa-miR-941	5557	6578	Embryonic stem cells		
hsa-miR-942	5558	6579		lung cancer	
hsa-miR-943	5559	6580	identified in Cervical cancer		
hsa-miR-944	5560	6581		various cancers (cervical, pancreatic, colonrectal)	
hsa-miR-95	5561	6582		various cancers (pancreatic, glioblastoma, colorectal etc)	
hsa-miR-9-5p	5562	6583	brain	Cancers and brain	

				disease	
hsa-miR-96-3p	5563	6584	stem cells	various cancers (prostate, lymphoma, HCC, etc) and inflammation	
hsa-miR-96-5p	5564	6585	stem cells	various cancers (prostate, lymphoma, HCC, etc) and inflammation	
hsa-miR-98-3p	5565	6586		various cancer cells	apoptosis
hsa-miR-98-5p	5566	6587		various cancer cells	apoptosis
hsa-miR-99a-3p	5567	6588	hemapoietic cells		
hsa-miR-99a-5p	5568	6589	hemapoietic cells		
hsa-miR-99b-3p	5569	6590	hemapoietic cells, embryonic stem cells		
hsa-miR-99b-5p	5570	6591	hemapoietic cells, embryonic stem cells		

[00339] MicroRNAs that are enriched in specific types of immune cells are listed in Table 11. Furthermore, novel miroRNAs are discovered in the immune cells in the art through micro-array hybridization and microtome analysis (Jima DD et al, Blood, 2010, 116:el 18-el27; Vaz C et al, BMC Genomics, 2010, 11,288, the content of each of which is incorporated herein by reference in its entirety). In Table 11, "HCC" represents hepatocellular carcinoma, "ALL" stands for acute lymphoblastsic leukemia and "CLL" stands for chrominc lymphocytic leukemia.

Table 11. microRNAs in immune cells

microRNA	**mir SEQ ID**	**BS SEQ ID**	**tissues/cells with MicroRNAs**	**associated diseases**	**biological functions/targe ts**
hsa-let-7a-2-3p	2508	3529	embryonic stem cells, lung, myeloid cells	inflammatory, various cancers (lung, cervical, breast, pancreatic, etc)	tumor suppressor, target to c-myc
hsa-let-7a-3p	2509	3530	embryonic stem cell, lung, myeloid cells	inflammatory, various cancers (lung, cervical, breast, pancreatic, etc)	tumor suppressor, target to c-myc
hsa-let-7a-5p	2510	3531	embryonic stem cells, lung,	inflammatory, various cancers	tumor suppressor,

			myeloid cells	(lung, cervical, breast, pancreatic, etc)	target to c-myc
hsa-let-7c	2513	3534	dendritic cells	various cacners (cervical, pancreatic, lung , esopphageal, etc)	tumor suppressor apoptosis (target to BCL-xl)
hsa-let-7e-3p	2516	3537	immune cells	various cancer cells, autoimmunity TLR signal pathway in endotoxin tolerance	tumor suppressor
hsa-let-7e-5p	2517	3538	immune cells	associated with various cancer cells	tumor suppressor
hsa-let-7f-1-3p	2518	3539	immune cells (T cells)	associated with various cancer cells	tumor suppressor
hsa-let-7f-2-3p	2519	3540	immune cells (T cells)	associated with various cancer cells	tumor suppressor
hsa-let-7f-5p	2520	3541	immune cells (T cells)	associated with various cancer cells	tumor suppressor
hsa-let-7g-3p	2521	3542	hematopoietic cells, adipose, smooth muscle cells	various cancer cells (lung, breast, etc)	tumor suppressor (target to NFkB, LOX1)
hsa-let-7g-5p	2522	3543	hematopoietic cells, adipose, smooth muscle cells	various cancer cells (lung, breast, etc)	tumor suppressor (target to NFkB, LOX1)
hsa-let-7i-3p	2523	3544	immune cells	chronic lymphocyte leukimia	tumor suppressor
hsa-let-7i-5p	2524	3545	immune cells	chronic lymphocyte leukimia	tumor suppressor
hsa-miR-10a-3p	2530	3551	hematopoeitic cells	acute myeoid leukemia	oncogene, cell growth
hsa-miR-10a-5p	2541	3562	hematopoietic cells	acute myeloid leukemia	oncogene, cell growth
hsa-miR-1184	2551	3572	Hematopoietic cells	downregulated in oral leukoplakia (OLK)	predited in the intron 22 of F8 gene
hsa-miR-125b-1-3p	2616	3637	hematopoietic cells (monocytes), brain (neuron)	various cancer (ALL, prostate, HCC, etc); TLR signal pathway in endotoxin tolerance	oncogene, cell differentiation
hsa-miR-125b-2-3p	2617	3638	hematopoietic cells (monocytes), brain (neuron)	various cancer (ALL, prostate, HCC etc); TLR signal pathway in endotoxin tolerance	oncogene cell differentiation
hsa-miR-125b-5p	2618	3639	hematopoietic cells, brain (neuron)	various cancer (Cutaneous T cell lymphomas, prostate, HCC, etc); TLR signal pathway	oncogene cell differentiation

				in endotoxin tolerance	
hsa-miR-1279	2652	3673	monocytes		
hsa-miR-130a-3p	2690	371 1	lung, monocytes, vascular endothelial cells	various cancers (basal cell carcinoma, HCC,ovarian, etc), drug resistance	pro-angiogenic
hsa-miR-1 30a-5p	2691	3712	lung, monocytes, vasscular endothelial cells	various cancers (basal cell carcinoma, HCC,ovarian, etc), drug resistance	pro-angiogenic
hsa-miR-132-3p	2697	371 8	brain(neuron), immune cells		
hsa-miR-132-5p	2699	3720	brain(neuron), immune cells		
hsa-miR- 142-3p	2720	3741	meyloid cells, hematopoiesis, APC cells		tumor suppressor, immune response
hsa-miR-142-5p	2721	3742	meyloid cells, hematopoiesis, APC cells		immune response
hsa-miR-143-5p	2723	3744	vascular smooth muscle, T-cells	increased in serum after virus infection	
hsa-miR-146a-3p	2730	375 1	immune cells, hematopoies is, ca rtilage,	associated with CLL, TLR signal pathway in endotoxin tolerance	
hsa-miR-146a-5p	273 1	3752	immune cells, hematopoiesis, cartilage,	associated with CLL, TLR signal pathway in endotoxin tolerance	
hsa-miR-146b-3p	2732	3753	immune cells	cancers (thyroid carcimona)	immune response
hsa-miR-146b-5p	2733	3754	embryoid body cells	thyroid cancer, associated with CLL	tumor invation, migration
hsa-miR-147a	2736	3757	Macrophage	inflammatory response	
hsa-miR- 147b	2737	3758	Macrophage	inflammatory response	
hsa-miR-148a-3p	2738	3759	hematopoietic cells	associated with CLL, T-lineage ALL	
hsa-miR-148a-5p	2739	3760	hematopoietic cells	associated with CLL, T-lineage ALL	
hsa-miR-150-3p	2744	3765	hematopoitic cells (lymphoid)	circulating plasma (acute myeloid leukemia)	
hsa-miR- 150-5p	2745	3766	hematopoitic cells (lymphoid)	circulating plasma (acute myeloid	

				leukemia)	
hsa-miR-15 1b	2748	3769	immune cells (B-cells)		
hsa-miR-1 55-3p	2756	3777	T/B cells, monocytes,breast	associated with CLL , TLR signal pathway in endotoxin tolerance ; upregulated in B cell lymphoma (CLL) and other cancers (breast, lung, ovarian, cervical, colorectal, prostate)	
hsa-miR-155-5p	2757	3778	T/B cells, monocytes,breast	associated with CLL , TLR signal pathway in endotoxin tolerance , upregulated in B cell lymphoma (CLL) and other cancers (breast, lung, ovarian, cervical, colorectal, prostate)	
hsa-miR- 15a-3p	2759	3780	blood, lymphocyte, hematopoietic tissues (spleen)	chronic lymphocytic leukemia	
hsa-miR-15a-5p	2760	3781	blood, lymphocyte, hematopoietic tissues (spleen)	chronic lymphocytic leukemia	
hsa-miR-15b-3p	276 1	3782	blood, lymphocyte, hematopoietic tissues (spleen)		cell cycle, proliferation
hsa-miR-15b-5p	2762	3783	blood, lymphocyte, hematopoietic tissues (spleen)		cell cycle, proliferation
hsa-miR-16-l-3p	2763	3784	embryonic stem cells, blood, hematopoietic tissues (spleen)	chronic lymphocytic leukemia	
hsa-miR- 16-2-3p	2764	3785	blood, lymphocyte, hematopoietic tissues (spleen)		
hsa-miR-16-5p	2765	3786	blood, lymphocyte, hematopoietic tissues		
hsa-miR-1 81a-3p	2769	3790	glioblast, myeloid cells,		

			Embryonic stem cells		
hsa-miR-181a-5p	2770	3791	glioblast, myeloid cells, Embryonic stem cells		
hsa-miR-182-3p	2776	3797	immune cells	colonrectal cancer, autoimmne	immune response
hsa-miR-182-5p	2778	3799	lung, immune cells	autoimmune	immune response
hsa-miR-197-3p	2827	3848	blood (myeloid), other tissues	various cancers (thyroid tumor, leukemia, etc)	
hsa-miR-197-5p	2828	3849	blood (myeloid), other tissues	various cancers (thyroid tumor, leukemia, etc)	
hsa-miR-21-3p	2879	3099	glioblast, Blood (meyloid cells), liver, vascular endothelial cells	autoimmune, heart diseases, cancers	
hsa-miR-214-3p	2880	3901	immune cells, pancreas	varioua cancers (melanoma, pancreatic, ovarian)	immune response
hsa-miR-214-5p	2881	3902	immune cells, pancreas	varioua cancers (melanoma, pancreatic, ovarian)	immune response
hsa-miR-21-5p	2883	3904	blood (myeloid cells), liver, endothelial cells	autoimmune, heart diseases, cancers	
hsa-miR-221-3p	2894	3915	endothelial cells, immune cells	breast cancer,upregulated in thyroid cell transformation induced by HMGA1, TLR signal pathway in endotoxin tolerance, upregulated in T cell ALL	angiogenesis/vasculogenesis
hsa-miR-221-5p	2895	3916	endothelial cells, immune cells	breast cancer,upregulated in thyroid cell transformation induced by HMGA1, TLR signal pathway in endotoxin tolerance , upregulated in T cell ALL	angiogenesis/vasculogenesis
hsa-miR-223-3p	2898	3919	meyloid cells	associated with CLL	
hsa-miR-223-5p	2899	3920	meyloid cells	associated with CLL	

hsa-miR-23b-3p	2913	3934	blood, myeloid cells	cancers (renal cancer, glioblastoma, prostate, etc) and autoimmune	
hsa-miR-23b-5p	2914	3935	blood, myeloid cells	cancers(glioblastoma, prostate, etc) and autoimmune	
hsa-miR-24-l-5p	2916	3937	lung, myeloid cells		
hsa-miR-24-2-5p	2917	3938	lung, myeloid cells		
hsa-miR-24-3p	2918	3939	lung, myeloid cells		
hsa-miR-26a-l-3p	2927	3948	embryonic stem cells, blood (T cells)	chronic lymphocyte leukemia and other cancers	cell cycle and differentiation
hsa-miR-26a-2-3p	2928	3949	blood (Tcells), other tissues	chronic lymphocyte leukemia and other cancers	cell cycle and differentiation
hsa-miR-26a-5p	2929	3950	blood (Tcells), other tissues	chronic lymphocyte leukemia and other cancers	cell cycle and differentiation
hsa-miR-26b-3p	2930	3951	hematopoietic cells		
hsa-miR-26b-5p	2931	3952	hematopoietic cells		
hsa-miR-27a-3p	2932	3953	myeloid_cells	various_cancer cells	
hsa-miR-27a-5p	2933	3954	myeloid_cells	various_cancer cells	
hsa-miR-27b-3p	2934	3955	myeloid cells, vascular endothelial_cells	various_cancer cells	pro-angiogenic_
hsa-miR-28-3p	2936	3957	blood(immune cells)_	B/T cell lymphoma_	
hsa-miR-28-5p	2937	3958	blood(immune cells)_	B/T cell lymphoma_	
hsa-miR-2909	2939	3960	T-Lymphocytes_		
hsa-miR-29a-3p	2948	3969	immuno system, colonrectun	various cancers, neurodegenative disease	tumor suppression, immune modulation (mir-29_family)_
hsa-miR-29a-5p	2949	3970	immuno system, colonrectun	various cancers, neurodegenative disease	adaptive immunity
hsa-miR-29b-l-5p	2950	3971	immuno system_	associated with CLL, other cancers, neurodegenative disease	adaptive immunity_
hsa-miR-29b-2-5p	2951	3972	immuno system_	associated with CLL, other cancers,	adaptive immunity_
hsa-miR-29b-3p	2952	3973	immuno system_	associated with CLL, other cancers	adaptive immunity_
hsa-miR-29c-3p	2953	3974	immuno system_	associated with	adaptive

				CLL, other cancers	immunity
hsa-miR-29c-5p	2954	3975	immuno system	associated with CLL, other cancers	adaptive immunity
hsa-miR-30e-3p	2984	4005	myeloid cells, glia cells		
hsa-miR-30e-5p	2985	4006	myeloid cells, glia cells		
hsa-miR-331-5p	3130	4151	lymphocytes		
hsa-miR-339-3p	3137	4158	immune cells		
hsa-miR-339-5p	3138	4159	immune cells		
hsa-miR-345-3p	3147	4168	hematopoietic cells	increased in follicular lymphoma(53), other cancers	
hsa-miR-345-5p	3148	4169	hematopoietic cells	increased in follicular lymphoma(53)	
hsa-miR-346	3149	4170	immume cells	cancers and autoimmune	
hsa-miR-34a-3p	3150	4171	breast, myeloid cells, ciliated epithelial cells	gastric cancer, CLL, other	tumor suppressor, p53 inducible
hsa-miR-34a-5p	3151	4172	breast, myeloid cells, ciliated epithelial cells	gastric cancer, CLL, other	tumor suppressor, p53 inducible
hsa-miR-363-3p	3193	4214	kidney stem cell, blood cells		
hsa-miR-363-5p	3194	4215	kidney stem cell, blood cells		
hsa-miR-372	3277	4298	hematopoietic cells, lung, placental (blood)		
hsa-miR-377-3p	3294	4315	hematopoietic cells		
hsa-miR-377-5p	3295	4316	hematopoietic cells		
hsa-miR-493-3p	4947	5968	myeloid cells, pancreas (islet)		
hsa-miR-493-5p	4948	5969	myeloid cells, pancreas (islet)		
hsa-miR-542-3p	5106	6127	monocytes		targets to survivin , introduce growth arrest
hsa-miR-548b-5p	5157	6178	immune cells frontal cortex		
hsa-miR-548c-5p	5159	6180	immune cells frontal cortex		
hsa-miR-548i	5168	6189	embryonic stem cells (41), immune cells		
hsa-miR-548j	5169	6190	immune cells		

hsa-miR-548n	5173	6194	embryonic stem cells , immune cells		
hsa-miR-574-3p	5279	6300	blood (myeloid cells)	increased in follicular lymphoma(53)	
hsa-miR-598	5310	6331	in blood lymphocytes (PBL)		
hsa-miR-935	5547	6568	identified in human cervical cancer blood mononuclear cells	associated with energy metabolism/obesity, medullablastoma/neural stem cells	
hsa-miR-99a-3p	5567	6588	hemapoietic cells		
hsa-miR-99a-5p	5568	6589	hemapoietic cells, plasma (exosome)		
hsa-miR-99b-3p	5569	6590	hemapoietic cells, Embryonic stem cells,		
hsa-miR-99b-5p	5570	6591	hemapoietic cells, Embryonic stem cells , plasma(exosome)		

III. Modifications

[00340] Herein, in a signal-sensor polynucleotide (such as a primary construct or a mRNA molecule), the terms "modification" or, as appropriate, "modified" refer to modification with respect to A, G, U or C ribonucleotides. Generally, herein, these terms are not intended to refer to the ribonucleotide modifications in naturally occurring 5'-terminal mRNA cap moieties. In a polypeptide, the term "modification" refers to a modification as compared to the canonical set of 20 amino acids.

[00341] The modifications may be various distinct modifications. In some embodiments, the coding region, the flanking regions and/or the terminal regions may contain one, two, or more (optionally different) nucleoside or nucleotide modifications. In some embodiments, a modified signal-sensor polynucleotide, primary construct, or mmRNA introduced to a cell may exhibit reduced degradation in the cell, as compared to an unmodified signal-sensor polynucleotide, primary construct, or mmRNA.

[00342] The signal-sensor polynucleotides, primary constructs, and mmRNA can include any useful modification, such as to the sugar, the nucleobase, or the

internucleoside linkage *(e.g.* to a linking phosphate / to a phosphodiester linkage / to the phosphodiester backbone). One or more atoms of a pyrimidine nucleobase may be replaced or substituted with optionally substituted amino, optionally substituted thiol, optionally substituted alkyl (e.g., methyl or ethyl), or halo (e.g., chloro or fluoro). In certain embodiments, modifications (e.g., one or more modifications) are present in each of the sugar and the internucleoside linkage. Modifications according to the present invention may be modifications of ribonucleic acids (RNAs) to deoxyribonucleic acids (DNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs) or hybrids thereof). Additional modifications are described herein.

[00343] As described herein, in some embodiments, the signal-sensor polynucleotides, primary constructs, and mmRNA of the invention do not substantially induce an innate immune response of a cell into which the mRNA is introduced. Featues of an induced innate immune response include 1) increased expression of pro-inflammatory cytokines, 2) activation of intracellular PRRs (RIG-I, MDA5, etc, and/or 3) termination or reduction in protein translation. In other embodiments, an immune response is induced.

[00344] In certain embodiments, it may desirable to intracellularly degrade a modified nucleic acid molecule introduced into the cell. For example, degradation of a modified nucleic acid molecule may be preferable if precise timing of protein production is desired. Thus, in some embodiments, the invention provides a modified nucleic acid molecule containing a degradation domain, which is capable of being acted on in a directed manner within a cell.

[00345] In another aspect, the present disclosure provides signal-sensor polynucleotides comprising a nucleoside or nucleotide that can disrupt the binding of a major groove interacting, e.g. binding, partner with the polynucleotide (e.g., where the modified nucleotide has decreased binding affinity to major groove interacting partner, as compared to an unmodified nucleotide).

[00346] The signal-sensor polynucleotides, primary constructs, and mmRNA can optionally include other agents (e.g., RNAi-inducing agents, RNAi agents, siRNAs, shRNAs, miRNAs, antisense RNAs, ribozymes, catalytic DNA, tRNA, RNAs that induce triple helix formation, aptamers, vectors, etc.). In some embodiments, the signal-sensor

polynucleotides, primary constructs, or mmRNA may include one or more messenger RNAs (mRNAs) and one or more modified nucleoside or nucleotides (e.g., mmRNA molecules). Details for these signal-sensor polynucleotides, primary constructs, and mmRNA follow.

Signal-sensor Polynucleotides and Primary Constructs

[00347] The signal-sensor polynucleotides, primary constructs, and mmRNA of the invention includes a first region of linked nucleosides encoding an oncology-related polypeptide of interest, a first flanking region located at the 5' terminus of the first region, and a second flanking region located at the 3' terminus of the first region.

[00348] In some embodiments, the signal-sensor polynucleotide, primary construct, or mmRNA are constructed according to the methods and modifications of International Application PCT/US12/058519 filed October 3, 2012 (M9), the contents of which are incorporated herein by reference in their entirety.

[00349] The signal-sensor polynucleotides, primary constructs, and mmRNA can optionally include 5' and/or 3' flanking regions, which are described herein.

Signal-sensor Modified RNA (mmRNA) Molecules

[00350] The present invention also includes the building blocks, e.g., modified ribonucleosides, modified ribonucleotides, of modified signal-sensor mRNA (mmRNA) molecules. For example, these building blocks can be useful for preparing the signal-sensor polynucleotides, primary constructs, or mmRNA of the invention. Such building blocks are taught in co-pending International Application PCT/US12/058519 filed October 3, 2012 (M9), the contents of which are incorporated herein by reference in their entirety.

Modifications on the Nucleobase

[00351] The present disclosure provides for modified nucleosides and nucleotides. As described herein "nucleoside" is defined as a compound containing a sugar molecule (e.g., a pentose or ribose) or a derivative thereof in combination with an organic base (e.g., a purine or pyrimidine) or a derivative thereof (also referred to herein as "nucleobase"). As described herein, "nucleotide" is defined as a nucleoside including a phosphate group. In some embodiments, the nucleosides and nucleotides described

herein are generally chemically modified on the major groove face. Exemplary non-limiting modifications include an amino group, a thiol group, an alkyl group, a halo group, or any described herein. The modified nucleotides may by synthesized by any useful method, as described herein (e.g., chemically, enzymatically, or recombinantly to include one or more modified or non-natural nucleosides).

[00352] The modified nucleosides and nucleotides can include a modified nucleobase. Examples of nucleobases found in RNA include, but are not limited to, adenine, guanine, cytosine, and uracil. Examples of nucleobase found in DNA include, but are not limited to, adenine, guanine, cytosine, and thymine. These nucleobases can be modified or wholly replaced to provide signal-sensor polynucleotides, primary constructs, or mmRNA molecules having enhanced properties. For example, the nucleosides and nucleotides described herein can be chemically modified. In some embodiments, chemical modifications can include an amino group, a thiol group, an alkyl group, or a halo group.

Modifications on the Internucleoside Linkage

[00353] The modified nucleotides, which may be incorporated into a signal-sensor polynucleotide, primary construct, or mmRNA molecule, can be modified on the internucleoside linkage (e.g., phosphate backbone). Herein, in the context of the polynucleotide backbone, the phrases "phosphate" and "phosphodiester" are used interchangeably. Backbone phosphate groups can be modified by replacing one or more of the oxygen atoms with a different substituent. Further, the modified nucleosides and nucleotides can include the wholesale replacement of an unmodified phosphate moiety with another internucleoside linkage as described herein. Examples of modified phosphate groups include, but are not limited to, phosphorothioate, phosphoroselenates, boranophosphates, boranophosphate esters, hydrogen phosphonates, phosphoramidates, phosphorodiamidates, alkyl or aryl phosphonates, and phosphotriesters. Phosphorodithioates have both non-linking oxygens replaced by sulfur. The phosphate linker can also be modified by the replacement of a linking oxygen with nitrogen (bridged phosphoramidates), sulfur (bridged phosphorothioates), and carbon (bridged methylene-phosphonates) .

[00354] The α-thio substituted phosphate moiety is provided to confer stability to RNA and DNA polymers through the unnatural phosphorothioate backbone linkages. Phosphorothioate DNA and RNA have increased nuclease resistance and subsequently a longer half-life in a cellular environment. Phosphorothioate linked signal-sensor polynucleotides, primary constructs, or mmRNA molecules are expected to also reduce the innate immune response through weaker binding/activation of cellular innate immune molecules.

[00355] In specific embodiments, a modified nucleoside includes an alpha-thio-nucleoside (e.g., 5'-0-(l-thiophosphate)-adenosine, 5'-0-(l-thiophosphate)-cytidine (a-thio-cytidine), 5'-0-(l-thiophosphate)-guanosine, 5'-0-(l-thiophosphate)-uridine, or 5'-0-(1-thiophosphate)-pseudouridine).

[00356] Other internucleoside linkages that may be employed according to the present invention, including internucleoside linkages which do not contain a phosphorous atom, are described herein below.

Combinations of Modified Sugars, Nucleobases, and Internucleoside Linkages

[00357] The signal-sensor polynucleotides, primary constructs, and mmRNA of the invention can include a combination of modifications to the sugar, the nucleobase, and/or the internucleoside linkage. These combinations can include any one or more modifications described herein or in International Application PCT/US12/058519 filed October 3, 2012 (M9), the contents of which are incorporated herein by reference in their entirety.

Synthesis of Signal-sensor primary constructs, and mmRNA Molecules

[00358] The signal-sensor polypeptides, primary constructs, and mmRNA molecules for use in accordance with the invention may be prepared according to any useful technique, as described herein. The modified nucleosides and nucleotides used in the synthesis of signal-sensor polynucleotides, primary constructs, and mmRNA molecules disclosed herein can be prepared from readily available starting materials using the following general methods and procedures. Where typical or preferred process conditions (e.g., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are provided, a skilled artisan would be able to optimize and develop additional process conditions. Optimum reaction conditions may vary with the particular

reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

[00359] The processes described herein can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (e.g., ^{1}H or ^{13}C) infrared spectroscopy, spectrophotometry (e.g., UV-visible), or mass spectrometry, or by chromatography such as high performance liquid chromatography (HPLC) or thin layer chromatography.

[00360] Preparation of signal-sensor polynucleotides, primary constructs, and mmRNA molecules of the present invention can involve the protection and deprotection of various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups can be readily determined by one skilled in the art. The chemistry of protecting groups can be found, for example, in Greene, et al, *Protective Groups in Organic Synthesis*, 2d. Ed., Wiley & Sons, 1991, which is incorporated herein by reference in its entirety.

[00361] The reactions of the processes described herein can be carried out in suitable solvents, which can be readily selected by one of skill in the art of organic synthesis. Suitable solvents can be substantially nonreactive with the starting materials (reactants), the intermediates, or products at the temperatures at which the reactions are carried out, i.e., temperatures which can range from the solvent's freezing temperature to the solvent's boiling temperature. A given reaction can be carried out in one solvent or a mixture of more than one solvent. Depending on the particular reaction step, suitable solvents for a particular reaction step can be selected.

[00362] Resolution of racemic mixtures of modified nucleosides and nucleotides (e.g., mmRNA molecules) can be carried out by any of numerous methods known in the art. An example method includes fractional recrystallization using a "chiral resolving acid" which is an optically active, salt-forming organic acid. Suitable resolving agents for fractional recrystallization methods are, for example, optically active acids, such as the D and L forms of tartaric acid, diacetyltartaric acid, dibenzoyltartaric acid, mandelic acid, malic acid, lactic acid or the various optically active camphorsulfonic acids. Resolution of racemic mixtures can also be carried out by elution on a column packed with an

optically active resolving agent *(e.g.,* dinitrobenzoylphenylglycine). Suitable elution solvent composition can be determined by one skilled in the art.

[00363] Modified nucleosides and nucleotides (e.g., building block molecules) can be prepared according to the synthetic methods described in Ogata et al, J. Org. Chem. 74:2585-2588 (2009); Purmal et al, Nucl. Acids Res. 22(1): 72-78, (1994); Fukuhara et al, Biochemistry, 1(4): 563-568 (1962); and Xu et al, Tetrahedron, 48(9): 1729-1740 (1992), each of which are incorporated by reference in their entirety.

[00364] The signal-sensor polynucleotides, primary constructs, and mmRNA of the invention may or may not be uniformly modified along the entire length of the molecule. For example, one or more or all types of nucleotide *(e.g.,* purine or pyrimidine, or any one or more or all of A, G, U, C) may or may not be uniformly modified in a polynucleotide of the invention, or in a given predetermined sequence region thereof *(e.g.* one or more of the sequence regions represented in Figure 1). In some embodiments, all nucleotides X in a signal-sensor polynucleotide of the invention (or in a given sequence region thereof) are modified, wherein X may any one of nucleotides A, G, U, C, or any one of the combinations A+G, A+U, A+C, G+U, G+C, U+C, A+G+U, A+G+C, G+U+C or A+G+C.

[00365] Different sugar modifications, nucleotide modifications, and/or internucleoside linkages (e.g., backbone structures) may exist at various positions in the signal-sensor polynucleotide, primary construct, or mmRNA. One of ordinary skill in the art will appreciate that the nucleotide analogs or other modification(s) may be located at any position(s) of a signal-sensor polynucleotide, primary construct, or mmRNA such that the function of the signal-sensor polynucleotide, primary construct, or mmRNA is not substantially decreased. A modification may also be a 5' or 3' terminal modification. The signal-sensor polynucleotide, primary construct, or mmRNA may contain from about 1% to about 100% modified nucleotides (either in relation to overall nucleotide content, or in relation to one or more types of nucleotide, i.e. any one or more of A, G, U or C) or any intervening percentage (e.g., from 1% to 20%>, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from

20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100%).

[00366] In some embodiments, the signal-sensor polynucleotide, primary construct, or mmRNA includes a modified pyrimidine (e.g., a modified uracil/uridine/U or modified cytosine/cytidine/C). In some embodiments, the uracil or uridine (generally: U) in the signal-sensor polynucleotide, primary construct, or mmRNA molecule may be replaced with from about 1% to about 100% of a modified uracil or modified uridine (e.g., from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100% of a modified uracil or modified uridine). The modified uracil or uridine can be replaced by a compound having a single unique structure or by a plurality of compounds having different structures (e.g., 2, 3, 4 or more unique structures, as described herein). In some embodiments, the cytosine or cytidine (generally: C) in the signal-sensor polynucleotide, primary construct, or mmRNA molecule may be replaced with from about 1% to about 100% of a modified cytosine or modified cytidine (e.g., from 1% to 20%>, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%,

from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100% of a modified cytosine or modified cytidine). The modified cytosine or cytidine can be replaced by a compound having a single unique structure or by a plurality of compounds having different structures (e.g., 2, 3, 4 or more unique structures, as described herein).

Combinations of Nucleotides

[00367] Further examples of modified nucleotides and modified nucleotide combinations are provided in International Application PCT/US12/058519 filed October 3, 2012 (M9) the contents of which are incorporated herein by reference in their entirety.

[00368] In some embodiments, at least 25% of the cytidines are replaced (e.g., at least about 30%, at least about 35%, at least about 40%>, at least about 45%, at least about 50%>, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about **75%o,** at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100%).

[00369] In some embodiments, at least 25% of the uracils are replaced (e.g., at least about **30%o,** at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about **75%o,** at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100%).

[00370] In some embodiments, at least 25% of the cytidines are replaced, and at least **25%o** of the uracils are replaced (e.g., at least about 30%, at least about 35%, at least about **40%o,** at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100%).

IV. Pharmaceutical Compositions

Formulation, Administration, Delivery and Dosing

[00371] The present invention provides signal-sensor polynucleotides, primary constructs and mmRNA compositions and complexes in combination with one or more pharmaceutically acceptable excipients. Pharmaceutical compositions may optionally

comprise one or more additional active substances, e.g. therapeutically and/or prophylactically active substances. General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in *Remington: The Science and Practice of Pharmacy* 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference).

[00372] In some embodiments, compositions are administered to humans, human patients or subjects. For the purposes of the present disclosure, the phrase "active ingredient" generally refers to signal-sensor polynucleotides, primary constructs and mmRNA to be delivered as described herein.

[00373] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other animal, *e.g.,* to non-human animals, e.g. non-human mammals. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

[00374] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

[00375] A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical

composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[00376] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100%, e.g., between .5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

Formulations

[00377] The signal-sensor polynucleotide, primary construct, and mmRNA of the invention can be formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection; (3) permit the sustained or delayed release (e.g., from a depot formulation of the signal-sensor polynucleotide, primary construct, or mmRNA); (4) alter the biodistribution (e.g., target the polynucleotide, primary construct, or mmRNA to specific tissues or cell types); (5) increase the translation of encoded protein *in vivo;* and/or (6) alter the release profile of encoded protein in vivo. In addition to traditional excipients such as any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, excipients of the present invention can include, without limitation, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with signal-sensor polynucleotide, primary construct, or mmRNA (e.g., for transplantation into a subject), hyaluronidase, nanoparticle mimics and combinations thereof. Further, the signal-sensor polynucleotide, primary construct, or mmRNA of the present invention may be formulated using self-assembled nucleic acid nanoparticles.

[00378] Accordingly, the formulations of the invention can include one or more excipients, each in an amount that together increases the stability of the signal-sensor polynucleotide, primary construct, or mmRNA, increases cell transfection by the signal-

sensor polynucleotide, primary construct, or mmRNA, increases the expression of polynucleotide, primary construct, or mmRNA encoded protein, and/or alters the release profile of signal-sensor polynucleotide, primary construct, or mmRNA encoded proteins. Further, the primary construct and mmRNA of the present invention may be formulated using self-assembled nucleic acid nanoparticles.

[00379] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of associating the active ingredient with an excipient and/or one or more other accessory ingredients.

[00380] A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" refers to a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient may generally be equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage including, but not limited to, one-half or one-third of such a dosage.

[00381] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the present disclosure may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered. For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient.

[00382] In some embodiments, the formulations described herein may contain at least one signal-sensor mmRNA. As a non-limiting example, the formulations may contain 1, 2, 3, 4 or 5 signal-sensor mmRNA. In one embodiment the formulation may contain modified mRNA encoding proteins selected from categories such as, proteins. In one embodiment, the formulation contains at least three signal-sensor modified mRNA encoding oncology-related proteins. In one embodiment, the formulation contains at least five signal-sensor modified mRNA encoding oncology-related proteins.

[00383] Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes, but is not limited to, any and all

solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, and the like, as suited to the particular dosage form desired. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see Remington: The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro, Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference). The use of a conventional excipient medium may be contemplated within the scope of the present disclosure, except insofar as any conventional excipient medium may be incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition.

[00384] In some embodiments, the particle size of the lipid nanoparticle may be increased and/or decreased. The change in particle size may be able to help counter biological reaction such as, but not limited to, inflammation or may increase the biological effect of the signal-sensor modified mRNA delivered to mammals.

[00385] Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, surface active agents and/or emulsifiers, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients may optionally be included in the pharmaceutical formulations of the invention.

[00386] Pharmaceutical compositions of the present invention may comprise at least one adjuvant which may be a chemo-adjuvant. Non-limiting examples of chemo-adjuvants and delivery systems which comprises a chemo-adjuvant are described in International Patent Publication No. WO2013 134349, the contents of which is herein incorporated by reference in its entirety. The chemo-adjuvant may be bonded to, non-covalently bonded to or encapsulated within a delivery vehicle described herein.

Lipidoids

[00387] The synthesis of lipidoids has been extensively described and formulations containing these compounds are particularly suited for delivery of signal-sensor polynucleotides, primary constructs or mmRNA (see Mahon et al., Bioconjug Chem. 2010 21:1448-1454; Schroeder et al, J Intern Med. 2010 267:9-21; Akinc et al, Nat

Biotechnol. 2008 26:561-569; Love et al, Proc Natl Acad Sci U S A. 2010 107:1864-1869; Siegwart et al, Proc Natl Acad Sci U S A. 201 1 108:12996-3001; all of which are incorporated herein in their entireties).

[00388] While these lipidoids have been used to effectively deliver double stranded small interfering RNA molecules in rodents and non-human primates (see Akinc et al., Nat Biotechnol. 2008 26:561-569; Frank-Kamenetsky et al, Proc Natl Acad Sci U S A. 2008 105:1 1915-1 1920; Akinc et al, Mol Ther. 2009 17:872-879; Love et al, Proc Natl Acad Sci U S A. 2010 107:1864-1869; Leuschner et al, Nat Biotechnol. 201 1 29:1005-1010; all of which is incorporated herein in their entirety), the present disclosure describes their formulation and use in delivering single stranded signal-sensor polynucleotides, primary constructs, or mmRNA. Complexes, micelles, liposomes or particles can be prepared containing these lipidoids and therefore, can result in an effective delivery of the signal-sensor polynucleotide, primary construct, or mmRNA, as judged by the production of an encoded protein, following the injection of a lipidoid formulation via localized and/or systemic routes of administration. Lipidoid complexes of signal-sensor polynucleotides, primary constructs, or mmRNA can be administered by various means including, but not limited to, intravenous, intramuscular, or subcutaneous routes.

[00389] *In vivo* delivery of nucleic acids may be affected by many parameters, including, but not limited to, the formulation composition, nature of particle PEGylation, degree of loading, oligonucleotide to lipid ratio, and biophysical parameters such as particle size (Akinc et al., Mol Ther. 2009 17:872-879; herein incorporated by reference in its entirety). As an example, small changes in the anchor chain length of poly(ethylene glycol) (PEG) lipids may result in significant effects on in vivo efficacy. Formulations with the different lipidoids, including, but not limited to penta[3-(l-laurylaminopropionyl)]-triethylenetetramine hydrochloride (TETA-5LAP; aka 98N12-5, see Murugaiah et al., Analytical Biochemistry, 401:61 (2010)), C12-200 (including derivatives and variants), and MD1, can be tested for *in vivo* activity.

[00390] The lipidoid referred to herein as "98N12-5" is disclosed by Akinc et al, Mol Ther. 2009 17:872-879 and is incorporated by reference in its entirety.

[00391] The lipidoid referred to herein as "C12-200" is disclosed by Love et al, Proc Natl Acad Sci U S A. 2010 107:1864-1869 and Liu and Huang, Molecular Therapy. 2010 669-670; both of which are herein incorporated by reference in their entirety. The lipidoid formulations can include particles comprising either 3 or 4 or more components in addition to signal-sensor polynucleotide, primary construct, or mmRNA. As an example, formulations with certain lipidoids, include, but are not limited to, 98N12-5 and may contain 42% lipidoid, 48% cholesterol and 10% PEG (CI 4 alkyl chain length). As another example, formulations with certain lipidoids, include, but are not limited to, CI2-200 and may contain 50%> lipidoid, 10%> disteroylphosphatidyl choline, 38.5% cholesterol, and 1.5% PEG-DMG.

[00392] Combinations of different lipidoids may be used to improve the efficacy of signal-sensor polynucleotide, primary construct, or mmRNA directed protein production as the lipidoids may be able to increase cell transfection by the signal-sensor polynucleotide, primary construct, or mmRNA; and/or increase the translation of encoded oncology-related protein (see Whitehead et al., Mol. Ther. 201 1, 19:1688-1694, herein incorporated by reference in its entirety).

[00393] In some embodiments, the particle size of the lipid nanoparticle may be increased and/or decreased. The change in particle size may be able to help counter biological reaction such as, but not limited to, inflammation or may increase the biological effect of , the signal-sensor polynucleotide, primary construct, or mmRNA delivered to subjects.

Liposomes, Lipoplexes, and Lipid Nanoparticles

[00394] The signal-sensor polynucleotide, primary construct, and mmRNA of the invention can be formulated using one or more liposomes, lipoplexes, or lipid nanoparticles. In one embodiment, pharmaceutical compositions of signal-sensor polynucleotide, primary construct, or mmRNA include liposomes. Liposomes are artificially-prepared vesicles which may primarily be composed of a lipid bilayer and may be used as a delivery vehicle for the administration of nutrients and pharmaceutical formulations. Liposomes can be of different sizes such as, but not limited to, a multilamellar vesicle (MLV) which may be hundreds of nanometers in diameter and may contain a series of concentric bilayers separated by narrow aqueous compartments, a

small unicellular vesicle (SUV) which may be smaller than 50 nm in diameter, and a large unilamellar vesicle (LUV) which may be between 50 and 500 nm in diameter. Liposome design may include, but is not limited to, opsonins or ligands in order to improve the attachment of liposomes to unhealthy tissue or to activate events such as, but not limited to, endocytosis. Liposomes may contain a low or a high pH in order to improve the delivery of the pharmaceutical formulations.

[00395] The formation of liposomes may depend on the physicochemical characteristics such as, but not limited to, the pharmaceutical formulation entrapped and the liposomal ingredients , the nature of the medium in which the lipid vesicles are dispersed, the effective concentration of the entrapped substance and its potential toxicity, any additional processes involved during the application and/or delivery of the vesicles, the optimization size, polydispersity and the shelf-life of the vesicles for the intended application, and the batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products.

[00396] In one embodiment, pharmaceutical compositions described herein may include, without limitation, liposomes such as those formed from l,2-dioleyloxy -*N*,*N*-dimethylaminopropane (DODMA) liposomes, DiLa2 liposomes from Marina Biotech (Bothell, WA), l,2-dilinoleyloxy-3-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[l,3]-dioxolane (DLin-KC2-DMA), and MC3 (US20 100324 120; herein incorporated by reference in its entirety) and liposomes which may deliver small molecule drugs such as, but not limited to, DOXIL® from Janssen Biotech, Inc. (Horsham, PA).

[00397] In one embodiment, pharmaceutical compositions described herein may include, without limitation, liposomes such as those formed from the synthesis of stabilized plasmid-lipid particles (SPLP) or stabilized nucleic acid lipid particle (SNALP) that have been previously described and shown to be suitable for oligonucleotide delivery in vitro and in vivo (see Wheeler et al. Gene Therapy. 1999 6:271-281; Zhang et al. Gene Therapy. 1999 6:1438-1447; Jeffs et al. Pharm Res. 2005 22:362-372; Morrissey et al, Nat Biotechnol. 2005 2:1002-1007; Zimmermann et al, Nature. 2006 441:1 11-1 14; Heyes et al. J Contr Rel. 2005 107:276-287; Semple et al. Nature Biotech. 2010 28:172-176; Judge et al. J Clin Invest. 2009 119:661-673; deFougerolles *Hum Gene Ther.* 2008

19: 125-132; all of which are incorporated herein in their entireties.) The original manufacture method by Wheeler et al. was a detergent dialysis method, which was later improved by Jeffs et al. and is referred to as the spontaneous vesicle formation method. The liposome formulations are composed of 3 to 4 lipid components in addition to the signal-sensor polynucleotide, primary construct, or mmRNA. As an example a liposome can contain, but is not limited to, 55% cholesterol, 20%> disteroylphosphatidyl choline (DSPC), 10% PEG-S-DSG, and 15% l,2-dioleyloxy -*N*,*N* -dimethylaminopropane (DODMA), as described by Jeffs et al. As another example, certain liposome formulations may contain, but are not limited to, 48% cholesterol, 20% DSPC, 2% PEG-c-DMA, and 30% cationic lipid, where the cationic lipid can be 1,2-distearloxy-*N*,*N*-dimethylaminopropane (DSDMA), DODMA, DLin-DMA, or l,2-dilinolenyloxy-3-dimethylaminopropane (DLenDMA), as described by Heyes et al.

[00398] In one embdodiment, pharmaceutical compsotions may include liposomes which may be formed to deliver signal-sensor mmRNA which may encode at least one immunogen. The mmRNA may be encapsulated by the liposome and/or it may be contained in an acqueous core which may then be encapsulated by the liposome (see International Pub. Nos. WO2012031046, WO2012031043, WO201203091 and WO2012006378 herein incorporated by reference in their entireties). In another embodiment, the signal-sensor mmRNA which may encode an immunogen may be formulated in a cationic oil-in-water emulstion where the emulsion particle comprises an oil core and a cationic lipid which can interact with the signal-sensor mmRNA anchoring the molecule to the emulsion particle (see International Pub. No. WO2012006380). In yet another embodiment, the lipid formulation may include at least cationic lipid, a lipid which may enhance transfection and a least one lipid which contains a hydrophilic head group linked to a lipid moiety (International Pub. No. WO201 1076807 and U.S. Pub. No. 201 10200582; herein incorporated by reference in their entireties). In another embodiment, the signal-sensor polynucleotides, primary constructs and/or mmRNA encoding an immunogen may be formulated in a lipid vesicle which may have crosslinks between functionalized lipid bilayers (see U.S. Pub. No. 20120177724, herein incorporated by reference in its entirety).

[00399] In one embodiment, the signal-sensor polynucleotides, primary constructs and/or mmRNA may be formulated in a lipid vesicle which may have crosslinks between functionalized lipid bilayers.

[00400] In one embodiment, the signal-sensor polynucleotides, primary constructs and/or mmRNA may be formulated in a lipid-polycation complex. The formation of the lipid-polycation complex may be accomplished by methods known in the art and/or as described in U.S. Pub. No. 20120178702, herein incorporated by reference in its entirety. As a non-limiting example, the polycation may include a cationic peptide or a polypeptide such as, but not limited to, polylysine, polyornithine and/or polyarginine. In another embodiment, the signal-sensor polynucleotides, primary constructs and/or mmRNA may be formulated in a lipid-polycation complex which may further include a neutral lipid such as, but not limited to, cholesterol or dioleoyl phosphatidylethanolamme (DOPE).

[00401] The liposome formulation may be influenced by, but not limited to, the selection of the cationic lipid component, the degree of cationic lipid saturation, the nature of the PEGylation, ratio of all components and biophysical parameters such as size. In one example by Semple et al. (Semple et al. Nature Biotech. 2010 28:172-176), the liposome formulation was composed of 57.1 % cationic lipid, 7.1% dipalmitoylphosphatidylcholme, 34.3 % cholesterol, and 1.4% PEG-c-DMA. As another example, changing the composition of the cationic lipid could more effectively deliver siRNA to various antigen presenting cells (Basha et al. Mol Ther. 201 1 19:2186-2200; herein incorporated by reference in its entirety).

[00402] In some embodiments, the ratio of PEG in the LNP formulations may be increased or decreased and/or the carbon chain length of the PEG lipid may be modified from C14 to CI8 to alter the pharmacokinetics and/or biodistribution of the LNP formulations. As a non-limiting example, LNP formulations may contain 1-5% of the lipid molar ratio of PEG-c-DOMG as compared to the cationic lipid, DSPC and cholesterol. In another embodiment the PEG-c-DOMG may be replaced with a PEG lipid such as, but not limited to, PEG- DSG (1,2-Distearoyl-sn-glycerol, methoxypolyethylene glycol) or PEG-DPG (1,2-Dipalmitoyl-sn-glycerol, methoxypolyethylene glycol). The

cationic lipid may be selected from any lipid known in the art such as, but not limited to, DLin-MC3-DMA, DLin-DMA, C12-200 and DLin-KC2-DMA.

[00403] In one embodiment, the LNP formulations of the signal-sensor polynucleotides, primary constructs and/or mmRNA may contain PEG-c-DOMG 3% lipid molar ratio. In another embodiment, the LNP formulations of the signal-sensor polynucleotides, primary constructs and/or mmRNA may contain PEG-c-DOMG 1.5% lipid molar ratio.

[00404] In one embodiment, the pharmaceutical compositions of the signal-sensor polynucleotides, primary constructs and/or mmRNA may include at least one of the PEGylated lipids described in International Publication No. 2012099755, herein incorporated by reference.

[00405] In one embodiment, the pharmaceutical compositions may be formulated in liposomes such as, but not limited to, DiLa2 liposomes (Marina Biotech, Bothell, WA), SMARTICLES® (Marina Biotech, Bothell, WA), neutral DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) based liposomes (e.g., siRNA delivery for ovarian cancer (Landen et al. Cancer Biology & Therapy 2006 5(12)1708-1713)) and hyaluronan-coated liposomes (Quiet Therapeutics, Israel).

[00406] In some embodiments the liposome may be a liposomal nanostructure which has been formulated for treatment of cancers and other diseases or to control the cholesterol metabolism in cells. The liposome nanostructure may also comprise a scavenger receptor type B-l (SR-B1) in order to kill cancer cells. Non-limiting examples of liposomal nanostructures, which may be used with the signal-sensor polynucleotides described herein, are described in International Publication No. WO2013126776, the contents of which are herein incorporated by reference in its entirety.

[00407] In one embodiment, the liposomes described herein may comprise at least one immunomodulator such as, but not limited to, cytokines. Formulations and methods of using the liposomes comprising at least one immunomodulator are described in International Publication No WO2013129935 and WO2013129936, the contents of each of which are herein incorporated by reference in their entirety. As a non-limiting example, the liposomes comprising at least one immunomodulator may be used in the treatment of cancer. The liposomes comprising an immunomodulator may comprise a

signal-sensor polynucleotide described herein. As a non-limiting example, the liposome comprising an immunomodulator may be used in a combination with at least one antibody such as the particulate or vesicular immunomodulators described in International Publication No WO2013129936, the contents of which are herein incorporated by reference in its entirety.

[00408] Lipid nanoparticle formulations may be improved by replacing the cationic lipid with a biodegradable cationic lipid which is known as a rapidly eliminated lipid nanoparticle (reLNP). Ionizable cationic lipids, such as, but not limited to, DLinDMA, DLin-KC2-DMA, and DLin-MC3-DMA, have been shown to accumulate in plasma and tissues over time and may be a potential source of toxicity. The rapid metabolism of the rapidly eliminated lipids can improve the tolerability and therapeutic index of the lipid nanoparticles by an order of magnitude from a 1 mg/kg dose to a 10 mg/kg dose in rat. Inclusion of an enzymatically degraded ester linkage can improve the degradation and metabolism profile of the cationic component, while still maintaining the activity of the reLNP formulation. The ester linkage can be internally located within the lipid chain or it may be terminally located at the terminal end of the lipid chain. The internal ester linkage may replace any carbon in the lipid chain.

[00409] In one embodiment, the internal ester linkage may be located on either side of the saturated carbon.

[00410] In one embodiment, an immune response may be elicited by delivering a lipid nanoparticle which may include a nanospecies, a polymer and an immunogen. (U.S. Publication No. 20120189700 and International Publication No. WO2012099805; herein incorporated by reference in their entireties). The polymer may encapsulate the nanospecies or partially encapsulate the nanospecies. The immunogen may be a recombinant oncology-related protein, a signal-sensor modified RNA and/or a primary construct described herein. In one embodiment, the lipid nanoparticle may be formulated for use in a vaccine such as, but not limited to, against a pathogen.

[00411] Lipid nanoparticles may be engineered to alter the surface properties of particles so the lipid nanoparticles may penetrate the mucosal barrier. Mucus is located on mucosal tissue such as, but not limted to, oral (e.g., the buccal and esophageal membranes and tonsil tissue), ophthalmic, gastrointestinal (e.g., stomach, small intestine,

large intestine, colon, rectum), nasal, respiratory (e.g., nasal, pharyngeal, tracheal and bronchial membranes), genital (e.g., vaginal, cervical and urethral membranes). Nanoparticles larger than 10-200 nm which are preferred for higher drug encapsulation efficiency and the ability to provide the sustained delivery of a wide array of drugs have been thought to be too large to rapidly diffuse through mucosal barriers. Mucus is continuously secreted, shed, discarded or digested and recycled so most of the trapped particles may be removed from the mucosla tissue within seconds or within a few hours. Large polymeric nanoparticles (200nm -500nm in diameter) which have been coated densely with a low molecular weight polyethylene glycol (PEG) diffused through mucus only 4 to 6-fold lower than the same particles diffusing in water (Lai et al. PNAS 2007 104(5): 1482-487; Lai et al. Adv Drug Deliv Rev. 2009 61(2): 158-171; herein incorporated by reference in their entirety). The transport of nanoparticles may be determined using rates of permeation and/or fluorescent microscopy techniques including, but not limited to, fluorescence recovery after photobleaching (FRAP) and high resolution multiple particle tracking (MPT).

[00412] The lipid nanoparticle engineered to penetrate mucus may comprise a polymeric material (i.e. a polymeric core) and/or a polymer-vitamin conjugate and/or a tri-block co-polymer. The polymeric material may include, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, poly(styrenes), polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyeneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. The polymeric material may be biodegradable and/or biocompatible. Non-limiting examples of specific polymers include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacralate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers),

polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate, polyoxymethylene, poloxamers, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), and trimethylene carbonate, polyvinylpyrrolidone. The lipid nanoparticle may be coated or associated with a co-polymer such as, but not limited to, a block co-polymer, and (poly(ethylene glycol))-(poly(propylene oxide))-(poly(ethylene glycol)) triblock copolymer (see US Publication 20120121718 and US Publication 20100003337; herein incorporated by reference in their entireties). The co-polymer may be a polymer that is generally regarded as safe (GRAS) and the formation of the lipid nanoparticle may be in such a way that no new chemical entities are created. For example, the lipid nanoparticle may comprise poloxamers coating PLGA nanoparticles without forming new chemical entities which are still able to rapidly penetrate human mucus (Yang et al. Angew. Chem. Int. Ed. 201 1 50:2597-2600; herein incorporated by reference in its entirety).

[00413] The vitamin of the polymer-vitamin conjugate may be vitamin E. The vitamin portion of the conjugate may be substituted with other suitable components such as, but not limited to, vitamin A, vitamin E, other vitamins, cholesterol, a hydrophobic moiety, or a hydrophobic component of other surfactants (e.g., sterol chains, fatty acids, hydrocarbon chains and alkylene oxide chains).

[00414] The lipid nanoparticle engineered to penetrate mucus may include surface altering agents such as, but not limited to, signal-sensor mmRNA, anionic protein (e.g., bovine serum albumin), surfactants (e.g., cationic surfactants such as for example dimethyldioctadecyl-ammonium bromide), sugars or sugar derivatives (e.g., cyclodextrin), nucleic acids, polymers (e.g., heparin, polyethylene glycol and poloxamer), mucolytic agents (e.g., N-acetylcysteine, mugwort, bromelain, papain, clerodendrum, acetylcysteine, bromhexine, carbocisteine, eprazinone, mesna, ambroxol, sobrerol, domiodol, letosteine, stepronin, tiopronin, gelsolin, thymosin β4 dornase alfa, neltenexine, erdosteine) and various DNases including rhDNase.. The surface altering agent may be embedded or enmeshed in the particle's surface or disposed (e.g., by coating, adsorption, covalent linkage, or other process) on the surface of the lipid nanoparticle. (see US Publication 20100215580 and US Publication 20080166414; herein incorporated by reference in their entireties).

[00415] The mucus penetrating lipid nanoparticles may comprise at least one signal-sensor mmRNA described herein. The signal-sensor mmRNA may be encapsulated in the lipid nanoparticle and/or disposed on the surface of the paricle. The signal-sensor mmRNA may be covalently coupled to the lipid nanoparticle. Formulations of mucus penetrating lipid nanoparticles may comprise a plurality of nanoparticles. Further, the formulations may contain particles which may interact with the mucus and alter the structural and/or adhesive properties of the surrounding mucus to decrease mucoadhesion which may increase the delivery of the mucus penetrating lipid nanoparticles to the mucosal tissue.

[00416] Lipid nanoparticles may be engineered to alter the surface properties of particles so the lipid nanoparticles may penetrate the mucosal barrier. Mucus is located on mucosal tissue such as, but not limted to, oral (e.g., the buccal and esophageal membranes and tonsil tissue), ophthalmic, gastrointestinal (e.g., stomach, small intestine, large intestine, colon, rectum), nasal, respiratory (e.g., nasal, pharyngeal, tracheal and bronchial membranes), genital (e.g., vaginal, cervical and urethral membranes). Nanoparticles larger than 10-200 nm which are preferred for higher drug encapsulation efficiency and the ability to provide the sustained delivery of a wide array of drugs have been thought to be too large to rapidly diffuse through mucosal barriers. Mucus is

continuously secreted, shed, discarded or digested and recycled so most of the trapped particles may be removed from the mucosla tissue within seconds or within a few hours. Large polymeric nanoparticles (200nm -500nm in diameter) which have been coated densely with a low molecular weight polyethylene glycol (PEG) diffused through mucus only 4 to 6-fold lower than the same particles diffusing in water (Lai et al. PNAS 2007 104(5): 1482-487; Lai et al. Adv Drug Deliv Rev. 2009 61(2): 158-171; herein incorporated by reference in their entirety). The transport of nanoparticles may be determined using rates of permeation and/or fluorescent microscopy techniques including, but not limited to, fluorescence recovery after photobleaching (FRAP) and high resolution multiple particle tracking (MPT).

[00417] The lipid nanoparticle engineered to penetrate mucus may comprise a polymeric material (i.e. a polymeric core) and/or a polymer-vitamin conjugate and/or a tri-block co-polymer. The polymeric material may including, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, poly(styrenes), polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyeneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. The polymeric material may be biodegradable and/or biocompatible. Non-limiting examples of specific polymers include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacralate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes,

derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate, polyoxymethylene, poloxamers, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), and trimethylene carbonate, polyvinylpyrrolidone. The lipid nanoparticle may be coated or associated with a co-polymer such as, but not limited to, a block co-polymer, and (poly(ethylene glycol))-(poly(propylene oxide))-(poly(ethylene glycol)) triblock copolymer (see US Publication 20120121718 and US Publication 20100003337; herein incorporated by reference in their entireties).

[00418] The vitamin of the polymer-vitamin conjugate may be vitamin E. The vitamin portion of the conjugate may be substituted with other suitable components such as, but not limited to, vitamin A, vitamin E, other vitamins, cholesterol, a hydrophobic moiety, or a hydrophobic component of other surfactants (e.g., sterol chains, fatty acids, hydrocarbon chains and alkylene oxide chains).

[00419] The lipid nanoparticle engineered to penetrate mucus may include surface altering agents such as, but not limited to, mmRNA, anionic protein (e.g., bovine serum albumin), surfactants (e.g., cationic surfactants such as for example dimethyldioctadecyl-ammonium bromide), sugars or sugar derivatives (e.g., cyclodextrin), nucleic acids, polymers (e.g., heparin, polyethylene glycol and poloxamer), mucolytic agents (e.g., N-acetylcysteine, mugwort, bromelain, papain, clerodendrum, acetylcysteine, bromhexine, carbocisteine, eprazinone, mesna, ambroxol, sobrerol, domiodol, letosteine, stepronin, tiopronin, gelsolin, thymosin β4 dornase alfa, neltenexine, erdosteine) and various DNases including rhDNase.. The surface altering agent may be embedded or enmeshed in the particle's surface or disposed (e.g., by coating, adsorption, covalent linkage, or other process) on the surface of the lipid nanoparticle. (see US Publication 20100215580 and US Publication 20080166414; herein incorporated by reference in their entireties).

[00420] The mucus penetrating lipid nanoparticles may comprise at least one signal-sensor polynucleotide, primary construct, or mmRNA described herein. The signal-sensor polynucleotide, primary construct, or mmRNA may be encapsulated in the lipid nanoparticle and/or disposed on the surface of the paricle. The signal-sensor polynucleotide, primary construct, or mmRNA may be covalently coupled to the lipid nanoparticle. Formulations of mucus penetrating lipid nanoparticles may comprise a plurality of nanoparticles. Further, the formulations may contain particles which may interact with the mucus and alter the structural and/or adhesive properties of the surrounding mucus to decrease mucoadhesion which may increase the delivery of the mucus penetrating lipid nanoparticles to the mucosal tissue.

[00421] In one embodiment, the nanoparticle may be for a dual modality therapy such as described by Mieszawska et al. (Bioconjugate Chemistry, 2013, 24 (9), pp 1429-1434; the contents of which is herein incorporated by reference in its entirety) comprising at least one therapeutic agent (e.g., a signal-sequence polynucleotide described herein). The therapeutic agent or agents formulated in the lipid nanoparticle may be an anti-angiogenic and a cytotoxic agent (see e.g., the polymer-lipid nanoparticles taught by Mieszawska et al. Bioconjugate Chemistry, 2013, 24 (9), pp 1429-1434; the contents of which is herein incorporated by reference in its entirety).

[00422] In another embodiment, the nanoparticle may comprise a LyP-1 peptide such as the nanocarrier composition described in International Patent Publication No. WO2013 100869, the contents of which are herein incorporated by reference in its entirety. The LyP-1 peptide may be contained in the nanoparticles disclosed herein, or may be a conjugate, derivative, analogue or pegylated form of the peptide. In one embodiment, a nanoparticle comprising the LyP-1 peptide may comprise a signal-sensor polynucleotide and may be used for cancer treatment and/or imaging.

[00423] In one embodiment, the signal-sensor polynucleotide, primary construct, or mmRNA is formulated as a lipoplex, such as, without limitation, the ATUPLEX™ system, the DACC system, the DBTC system and other siRNA-lipoplex technology from Silence Therapeutics (London, United Kingdom), STEMFECT™ from STEMGENT® (Cambridge, MA), and polyethylenimine (PEI) or protamine-based targeted and non-targeted delivery of nucleic acids acids (Aleku et al. Cancer Res. 2008 68:9788-9798;

Strumberg et al. Int J Clin Pharmacol Ther 2012 50:76-78; Santel et al, Gene Ther 2006 13:1222-1234; Santel et al, Gene Ther 2006 13:1360-1370; Gutbier et al, Pulm Pharmacol. Ther. 2010 23:334-344; Kaufmann et al. Microvasc Res 2010 80:286-293Weide et al. J Immunother. 2009 32:498-507; Weide et al. J Immunother. 2008 31:180-188; Pascolo Expert Opin. Biol. Ther. 4:1285-1294; Fotin-Mleczek et al, 201 1 J. Immunother. 34:1-15; Song et al, Nature Biotechnol. 2005, 23:709-717; Peer et al, Proc Natl Acad Sci U S A. 2007 6;104:4095-4100; deFougerolles *Hum Gene Ther.* 2008 19:125-132; all of which are incorporated herein by reference in its entirety).

[00424] In one embodiment such formulations may also be constructed or compositions altered such that they passively or actively are directed to different cell types *in vivo,* including but not limited to hepatocytes, immune cells, tumor cells, endothelial cells, antigen presenting cells, and leukocytes (Akinc et al. Mol Ther. 2010 18:1357-1364; Song et al, Nat Biotechnol. 2005 23:709-717; Judge et al, J Clin Invest. 2009 119:661-673; Kaufmann et al, Microvasc Res 2010 80:286-293; Santel et al, Gene Ther 2006 13:1222-1234; Santel et al, Gene Ther 2006 13:1360-1370; Gutbier et al, Pulm Pharmacol. Ther. 2010 23:334-344; Basha et al, Mol. Ther. 201 1 19:2186-2200; Fenske and Cullis, Expert Opin Drug Deliv. 2008 5:25-44; Peer et al, Science. 2008 319:627-630; Peer and Lieberman, Gene Ther. 201 1 18:1 127-1 133; all of which are incorporated herein by reference in its entirety). One example of passive targeting of formulations to liver cells includes the DLin-DMA, DLin-KC2-DMA and MC3-based lipid nanoparticle formulations which have been shown to bind to apolipoprotein E and promote binding and uptake of these formulations into hepatocytes in vivo (Akinc et al. Mol Ther. 2010 18:1357-1364; herein incorporated by reference in its entirety). Formulations can also be selectively targeted through expression of different ligands on their surface as exemplified by, but not limited by, folate, transferrin, N-acetylgalactosamine (GalNAc), and antibody targeted approaches (Kolhatkar et al., Curr Drug Discov Technol. 201 1 8:197-206; Musacchio and Torchilin, *Front Biosci.* 201 1 16:1388-1412; Yu et al, Mol Membr Biol. 2010 27:286-298; Patil et al, Crit Rev Ther Drug Carrier Syst. 2008 25:1-61; Benoit et al., Biomacromolecules. 201 1 12:2708-2714Zhao et al., Expert Opin Drug Deliv. 2008 5:309-319; Akinc et al, Mol Ther. 2010 18:1357-1364; Srinivasan et al, Methods Mol Biol. 2012 820:105-1 16; Ben-Arie et al, Methods Mol Biol. 2012 757:497-

507; Peer 2010 J Control Release. 20:63-68; Peer et al, Proc Natl Acad Sci U S A. 2007 104:4095-4100; Kim et al, Methods Mol Biol. 201 1 721:339-353; Subramanya et al, Mol Ther. 2010 18:2028-2037; Song et al, Nat Biotechnol. 2005 23:709-717; Peer et al, Science. 2008 319:627-630; Peer and Lieberman, Gene Ther. 201 1 18:1 127-1 133; all of which are incorporated herein by reference in its entirety)..

[00425] In one embodiment, the signal-sensor polynucleotide, primary construct, or mmRNA is formulated as a solid lipid nanoparticle. A solid lipid nanoparticle (SLN) may be spherical with an average diameter between 10 to 1000 nm. SLN possess a solid lipid core matrix that can solubilize lipophilic molecules and may be stabilized with surfactants and/or emulsifiers. In a further embodiment, the lipid nanoparticle may be a self-assembly lipid-polymer nanoparticle (see Zhang et al, ACS Nano, 2008, 2 (8), pp 1696-1702; herein incorporated by reference in its entirety).

[00426] Liposomes, lipoplexes, or lipid nanoparticles may be used to improve the efficacy of signal-sensor polynucleotide, primary construct, or mmRNA directed protein production as these formulations may be able to increase cell transfection by the signal-sensor polynucleotide, primary construct, or mmRNA; and/or increase the translation of encoded protein. One such example involves the use of lipid encapsulation to enable the effective systemic delivery of polyplex plasmid DNA (Heyes et al., Mol Ther. 2007 15:713-720; herein incorporated by reference in its entirety). The liposomes, lipoplexes, or lipid nanoparticles may also be used to increase the stability of the signal-sensor polynucleotide, primary construct, or mmRNA.

Polymers, Biodegradable Nanoparticles, and Core-Shell Nanoparticles

[00427] The signal-sensor polynucleotide, primary construct, and mmRNA of the invention can be formulated using natural and/or synthetic polymers. Non-limiting examples of polymers which may be used for delivery include, but are not limited to, Dynamic POLYCONJUGATE™ formulations from MIRUS® Bio (Madison, WI) and Roche Madison (Madison, WI), PHASERX™ polymer formulations such as, without limitation, SMARTT POLYMER TECHNOLOGY™ (Seattle, WA), DMRI/DOPE, poloxamer, VAXFECTIN® adjuvant from Vical (San Diego, CA), chitosan, cyclodextrin from Calando Pharmaceuticals (Pasadena, CA), dendrimers and poly(lactic-co-glycolic acid) (PLGA) polymers. RONDEL™ (RNAi/Oligonucleotide Nanoparticle Delivery)

polymers (Arrowhead Research Corporation, Pasadena, CA) and pH responsive co-block polymers such as, but not limited to, PHASERX™ (Seattle, WA).

[00428] A non-limiting example of PLGA formulations include, but are not limited to, PLGA injectable depots (e.g., ELIGARD® which is formed by dissolving PLGA in 66% N-methyl-2-pyrrolidone (NMP) and the remainder being aqueous solvent and leuprolide. Once injected, the PLGA and leuprolide peptide precipitates into the subcutaneous space).

[00429] Many of these polymer approaches have demonstrated efficacy in delivering oligonucleotides *in vivo* into the cell cytoplasm (reviewed in deFougerolles *Hum Gene Ther*. 2008 19:125-132; herein incorporated by reference in its entirety). Two polymer approaches that have yielded robust *in vivo* delivery of nucleic acids, in this case with small interfering RNA (siRNA), are dynamic polyconjugates and cyclodextrin-based nanoparticles. The first of these delivery approaches uses dynamic polyconjugates and has been shown *in vivo* in mice to effectively deliver siRNA and silence endogenous target mRNA in hepatocytes (Rozema et al, Proc Natl Acad Sci U S A. 2007 104:12982-12887). This particular approach is a multicomponent polymer system whose key features include a membrane-active polymer to which nucleic acid, in this case siRNA, is covalently coupled via a disulfide bond and where both PEG (for charge masking) and *N*-acetylgalactosamine (for hepatocyte targeting) groups are linked via pH-sensitive bonds (Rozema et al, Proc Natl Acad Sci U S A. 2007 104:12982-12887). On binding to the hepatocyte and entry into the endosome, the polymer complex disassembles in the low-pH environment, with the polymer exposing its positive charge, leading to endosomal escape and cytoplasmic release of the siRNA from the polymer. Through replacement of the ^-acetylgalactosamine group with a mannose group, it was shown one could alter targeting from asialoglycoprotein receptor-expressing hepatocytes to sinusoidal endothelium and Kupffer cells. Another polymer approach involves using transferrin-targeted cyclodextrin-containing polycation nanoparticles. These nanoparticles have demonstrated targeted silencing of the *EWS-FLI1* gene product in transferrin receptor-expressing Ewing's sarcoma tumor cells (Hu-Lieskovan *et al.,* Cancer Res.2005 65: 8984-8982) and siRNA formulated in these nanoparticles was well tolerated in non-human primates (Heidel *et al,* Proc Natl Acad Sci USA 2007 104:5715-21). Both of

these delivery strategies incorporate rational approaches using both targeted delivery and endosomal escape mechanisms.

[00430] The polymer formulation can permit the sustained or delayed release of signal-sensor polynucleotide, primary construct, or mmRNA (e.g., following intramuscular or subcutaneous injection). The altered release profile for the signal-sensor polynucleotide, primary construct, or mmRNA can result in, for example, translation of an encoded protein over an extended period of time. The polymer formulation may also be used to increase the stability of the signal-sensor polynucleotide, primary construct, or mmRNA. Biodegradable polymers have been previously used to protect nucleic acids other than mmRNA from degradation and been shown to result in sustained release of payloads in vivo (Rozema et al, Proc Natl Acad Sci U S A. 2007 104:12982-12887; Sullivan et al, Expert Opin Drug Deliv. 2010 7:1433-1446; Convertine et al., Biomacromolecules. 2010 Oct 1; Chu et al., Acc Chem Res. 2012 Jan 13; Manganiello et al., Biomaterials. 2012 33:2301-2309; Benoit et al, Biomacromolecules. 201 1 12:2708-2714; Singha et al, Nucleic Acid Ther. 201 1 2:133-147; deFougerolles Hum Gene Ther. 2008 19:125-132; Schaffert and Wagner, Gene Ther. 2008 16:1 131-1 138; Chaturvedi et al., Expert Opin Drug Deliv. 201 1 8:1455-1468; Davis, Mol Pharm. 2009 6:659-668; Davis, Nature 2010 464:1067-1070; herein incorporated by reference in its entirety).

[00431] In one embodiment, the pharmaceutical compositions may be sustained release formulations. In a further embodiment, the sustained release formulations may be for subcutaneous delivery. Sustained release formulations may include, but are not limited to, PLGA microspheres, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, FL), HYLENEX® (Halozyme Therapeutics, San Diego CA), surgical sealants such as fibrinogen polymers (Ethicon Inc. Cornelia, GA). TISSELL® (Baxter International, Inc Deerfield, IL), PEG-based sealants, and COSEAL® (Baxter International, Inc Deerfield, IL).

[00432] As a non-limiting example modified mRNA may be formulated in PLGA microspheres by preparing the PLGA microspheres with tunable release rates (e.g., days and weeks) and encapsulating the signal-sensor modified mRNA in the PLGA microspheres while maintaining the integrity of the signal-sensor modified mRNA during the encapsulation process. EVAc are non-biodegradeable, biocompatible polymers

which are used extensively in pre-clinical sustained release implant applications (e.g., extended release products Ocusert a pilocarpine ophthalmic insert for glaucoma or progestasert a sustained release progesterone intrauterine deivce; transdermal delivery systems Testoderm, Duragesic and Selegiline; catheters). Poloxamer F-407 NF is a hydrophilic, non-ionic surfactant triblock copolymer of polyoxyethylene-polyoxypropylene-polyoxyethylene having a low viscosity at temperatures less than 5°C and forms a solid gel at temperatures greater than 15°C. PEG-based surgical sealants comprise two synthetic PEG components mixed in a delivery device which can be prepared in one minute, seals in 3 minutes and is reabsorbed within 30 days. GELSITE® and natural polymers are capable of in-situ gelation at the site of administration. They have been shown to interact with protein and peptide therapeutic candidates through ionic ineraction to provide a stabilizing effect.

[00433] Polymer formulations can also be selectively targeted through expression of different ligands as exemplified by, but not limited by, folate, transferrin, and N-acetylgalactosamine (GalNAc) (Benoit et al., Biomacromolecules. 201 1 12:2708-2714; Rozema et al, Proc Natl Acad Sci U S A. 2007 104:12982-12887; Davis, Mol Pharm. 2009 6:659-668; Davis, Nature 2010 464:1067-1070; herein incorporated by reference in its entirety).

[00434] The signal-sensor mmRNA of the invention may be formulated with or in a polymeric compound. The polymer may include at least one polymer such as, but not limited to, polyethylene glycol (PEG), poly(l-lysine)(PLL), PEG grafted to PLL, cationic lipopolymer, biodegradable cationic lipopolymer, polyethyleneimine (PEI), cross-linked branched poly(alkylene imines), a polyamine derivative, a modified poloxamer, a biodegradable polymer, biodegradable block copolymer, biodegradable random copolymer, biodegradable polyester copolymer, biodegradable polyester block copolymer, biodegradable polyester block random copolymer, linear biodegradable copolymer, poly[a-(4-aminobutyl)-L-glycolic acid) (PAGA), biodegradable cross-linked cationic multi-block copolymers or combinations thereof .

[00435] As a non-limiting example, the signal-sensor mmRNA of the invention may be formulated with the polymeric compound of PEG grafted with PLL as described in U.S. Pat. No. 6,177,274 herein incorporated by reference in its entirety. The formulation may

be used for transfecting cells *in vitro* or for *in vivo* delivery of the signal-sensor mmRNA. In another example, the signal-sensor mmRNA may be suspended in a solution or medium with a cationic polymer, in a dry pharmaceutical composition or in a solution that is capable of being dried as described in U.S. Pub. Nos. 20090042829 and 20090042825 each of which are herein incorporated by reference in their entireties.

[00436] A polyamine derivative may be used to deliver nucleic acids or to treat and/or prevent a disease or to be included in an implantable or injectable device (U.S. Pub. No. 20100260817 herein incorporated by reference in its entirety). As a non-limiting example, a pharmaceutical composition may include the signal-sensor mmRNA and the polyamine derivative described in U.S. Pub. No. 20100260817 (the contents of which are incorporated herein by reference in its entirety.

[00437] For example, the signal-sensor mmRNA of the invention may be formulated in a pharmaceutical compound including a poly(alkylene imine), a biodegradable cationic lipopolymer, a biodegradable block copolymer, a biodegradable polymer, or a biodegradable random copolymer, a biodegradable polyester block copolymer, a biodegradable polyester polymer, a biodegradable polyester random copolymer, a linear biodegradable copolymer, PAGA, a biodegradable cross-linked cationic multi-block copolymer or combinations thereof. The biodegradable cationic lipopolymer may be made my methods known in the art and/or described in U.S. Pat. No. 6,696,038, U.S. App. Nos. 20030073619 and 20040142474 which is herein incorporated by reference in their entireties.The poly(alkylene imine) may be made using methods known in the art and/or as described in U.S. Pub. No. 20100004315, herein incorporated by reference in its entirety. The biodegradabale polymer, biodegradable block copolymer, the biodegradable random copolymer, biodegradable polyester block copolymer, biodegradable polyester polymer, or biodegradable polyester random copolymer may be made using methods known in the art and/or as described in U.S. Pat. Nos. 6,517,869 and 6,267,987, the contents of which are each incorporated herein by reference in its entirety. The linear biodegradable copolymer may be made using methods known in the art and/or as described in U.S. Pat. No. 6,652,886. The PAGA polymer may be made using methods known in the art and/or as described in U.S. Pat. Nos. 6,217,912 herein incorporated by reference in its entirety. The PAGA polymer may be copolymerized to

form a copolymer or block copolymer with polymers such as but not limited to, poly-L-lysine, polyargine, polyornithine, histones, avidin, protamines, polylactides and poly(lactide-co-glycolides). The biodegradable cross-linked cationic multi-block copolymers may be made my methods known in the art and/or as described in U.S. Pat. No. 8,057,821 or U.S. Pub. No. 2012009145 herein incorporated by reference in their entireties. For example, the multi-block copolymers may be synthesized using linear polyethyleneimine (LPEI) blocks which have distinct patterns as compared to branched polyethyleneimines. Further, the composition or pharmaceutical composition may be made by the methods known in the art, described herein, or as described in U.S. Pub. No. 20100004315 or U.S. Pat. Nos. 6,267,987 and 6,217,912 herein incorporated by reference in their entireties.

[00438] As described in U.S. Pub. No. 201000043 13, herein incorporated by reference in its entirety, a gene delivery composition may include a nucleotide sequence and a poloxamer. For example, the signal-sensor mmRNA of the present inveition may be used in a gene delivery composition with the poloxamer described in U.S. Pub. No. 20100004313.

[00439] In one embodiment, the polymer formulation of the present invention may be stabilized by contacting the polymer formulation, which may include a cationic carrier, with a cationic lipopolymer which may be covalently linked to cholesterol and polyethylene glycol groups. The polymer formulation may be contacted with a cationic lipopolymer using the methods described in U.S. Pub. No. 20090042829 herein incorporated by reference in its entirety. The cationic carrier may include, but is not limited to, polyethylenimine, poly(trimethylenimine), poly(tetramethylenimine), polypropylenimine, aminoglycoside-polyamine, dideoxy-diamino-b-cyclodextrin, spermine, spermidine, poly(2-dimethylamino)ethyl methacrylate, poly(lysine), poly(histidine), poly(arginine), cationized gelatin, dendrimers, chitosan, l,2-Dioleoyl-3-Trimethylammonium-Propane(DOTAP), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), l-[2-(oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM), 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-l-propanaminium trifluoroacetate (DOSPA), 3B-[N—(N',N'-Dimethylaminoethane)-carbamoyl]Cholesterol Hydrochloride

(DC-Cholesterol HC1) diheptadecylamidoglycyl spermidine (DOGS), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(l,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), N,N-dioleyl-N,N-dimethylammonium chloride DODAC) and combinations thereof.

[00440] The signal-sensor polynucleotide, primary construct, and mmRNA of the invention can also be formulated as a nanoparticle using a combination of polymers, lipids, and/or other biodegradable agents, such as, but not limited to, calcium phosphate. Components may be combined in a core-shell, hybrid, and/or layer-by-layer architecture, to allow for fine-tuning of the nanoparticle so to delivery of the signal-sensor polynucleotide, primary construct and mmRNA may be enhanced (Wang et al., Nat Mater. 2006 5:791-796; Fuller et al, Biomaterials. 2008 29:1526-1532; DeKoker et al, Adv Drug Deliv Rev. 201 1 63:748-761; Endres et al, Biomaterials. 201 1 32:7721-7731; Su et al, Mol Pharm. 201 1 Jun 6;8(3):774-87; herein incorporated by reference in its entirety).

[00441] Biodegradable calcium phosphate nanoparticles in combination with lipids and/or polymers have been shown to deliver signal-sensor polynucleotides, primary constructs and mmRNA *in vivo.* In one embodiment, a lipid coated calcium phosphate nanoparticle, which may also contain a targeting ligand such as anisamide, may be used to deliver the signal-sensor polynucleotide, primary construct and mmRNA of the present invention. For example, to effectively deliver siRNA in a mouse metastatic lung model a lipid coated calcium phosphate nanoparticle was used (Li et al., J Contr Rel. 2010 142: 416-421; Li et al, J Contr Rel. 2012 158:108-1 14; Yang et al, Mol Ther. 2012 20:609-615). This delivery system combines both a targeted nanoparticle and a component to enhance the endosomal escape, calcium phosphate, in order to improve delivery of the siRNA.

[00442] In one embodiment, calcium phosphate with a PEG-polyanion block copolymer may be used to deliver signal-sensor polynucleotides, primary constructs and mmRNA (Kazikawa et al, J Contr Rel. 2004 97:345-356; Kazikawa et al, J Contr Rel. 2006 111:368-370).

[00443] In one embodiment, a PEG-charge-conversional polymer (Pitella et al., Biomaterials. 201 1 32:3 106-3 114) may be used to form a nanoparticle to deliver the

signal-sensor polynucleotides, primary constructs and mmRNA of the present invention. The PEG-charge-conversional polymer may improve upon the PEG-polyanion block copolymers by being cleaved into a polycation at acidic pH, thus enhancing endosomal escape.

[00444] The use of core-shell nanoparticles has additionally focused on a high-throughput approach to synthesize cationic cross-linked nanogel cores and various shells (Siegwart et al, Proc Natl Acad Sci U S A. 201 1 108:12996-13001). The complexation, delivery, and internalization of the polymeric nanoparticles can be precisely controlled by altering the chemical composition in both the core and shell components of the nanoparticle. For example, the core-shell nanoparticles may efficiently deliver siRNA to mouse hepatocytes after they covalently attach cholesterol to the nanoparticle.

[00445] In one embodiment, a hollow lipid core comprising a middle PLGA layer and an outer neutral lipid layer containg PEG may be used to delivery of the signal-sensor polynucleotide, primary construct and mmRNA of the present invention. As a non-limiting example, in mice bearing a luciferease-expressing tumor, it was determined that the lipid-polymer-lipid hybrid nanoparticle significantly suppressed luciferase expression, as compared to a conventional lipoplex (Shi et al, Angew Chem Int Ed. 201 1 50:7027-7031).

Peptides and Proteins

[00446] The signal-sensor polynucleotide, primary construct, and mmRNA of the invention can be formulated with peptides and/or proteins in order to increase transfection of cells by the polynucleotide, primary construct, or mmRNA. In one embodiment, peptides such as, but not limited to, cell penetrating peptides and proteins and peptides that enable intracellular delivery may be used to deliver pharmaceutical formulations. A non-limiting example of a cell penetrating peptide which may be used with the pharmaceutical formulations of the present invention includes a cell-penetrating peptide sequence attached to polycations that facilitates delivery to the intracellular space, e.g., HIV-derived TAT peptide, penetratins, transportans, or hCT derived cell-penetrating peptides (see, e.g., Caron et al, Mol. Ther. 3(3):3 10-8 (2001); Langel, Cell-Penetrating Peptides: Processes and Applications (CRC Press, Boca Raton FL, 2002); El-Andaloussi et al, Curr. Pharm. Des. 11(28):3597-61 1 (2003); and Deshayes et al,

Cell. Mol. Life Sci. 62(16):1 839-49 (2005), all of which are incorporated herein by reference). The compositions can also be formulated to include a cell penetrating agent, e.g., liposomes, which enhance delivery of the compositions to the intracellular space. signal-sensor polynucleotides, primary constructs, and mmRNA of the invention may be complexed to peptides and/or proteins such as, but not limited to, peptides and/or proteins from Aileron Therapeutics (Cambridge, MA) and Permeon Biologies (Cambridge, MA) in order to enable intracellular delivery (Cronican et al., ACS Chem. Biol. 2010 5:747-752; McNaughton et al, Proc. Natl. Acad. Sci. USA 2009 106:61 11-61 16; Sawyer, Chem Biol Drug Des. 2009 73:3-6; Verdine and Hilinski, Methods Enzymol. 2012;503:3-33; all of which are herein incorporated by reference in its entirety).

[00447] In one embodiment, the cell-penetrating polypeptide may comprise a first domain and a second domain. The first domain may comprise a supercharged polypeptide. The second domain may comprise a protein-binding partner. As used herein, "protein-binding partner" includes, but are not limited to, antibodies and functional fragments thereof, scaffold proteins, or peptides. The cell-penetrating polypeptide may further comprise an intracellular binding partner for the protein-binding partner. The cell-penetrating polypeptide may be capable of being secreted from a cell where the signal-sensor polynucleotide, primary construct, or mmRNA may be introduced.

[00448] Formulations of the including peptides or proteins may be used to increase cell transfection by the signal-sensor polynucleotide, primary construct, or mmRNA, alter the biodistribution of the signal-sensor polynucleotide, primary construct, or mmRNA (e.g., by targeting specific tissues or cell types), and/or increase the translation of encoded protein.

Cells

[00449] The signal-sensor polynucleotide, primary construct, and mmRNA of the invention can be transfected *ex vivo* into cells, which are subsequently transplanted into a subject. As non-limiting examples, the pharmaceutical compositions may include red blood cells to deliver modified RNA to liver and myeloid cells, virosomes to deliver modified RNA in virus-like particles (VLPs), and electroporated cells such as, but not limited to, from MAXCYTE® (Gaithersburg, MD) and from ERYTECH® (Lyon, France) to deliver modified RNA. Examples of use of red blood cells, viral particles and

electroporated cells to deliver payloads other than mmRNA have been documented (Godfrin et al., Expert Opin Biol Ther. 2012 12:127-133; Fang et al, Expert Opin Biol Ther. 2012 12:385-389; Hu et al, Proc Natl Acad Sci U S A. 201 1 108:10980-10985; Lund et al, Pharm Res. 2010 27:400-420; Huckriede et al, J Liposome Res. 2007;17:39-47; Cusi, Hum Vaccin. 2006 2:1-7; de Jonge et al, Gene Ther. 2006 13:400-41 1; all of which are herein incorporated by reference in its entirety).

[00450] Cell-based formulations of the signal-sensor polynucleotide, primary construct, and mmRNA of the invention may be used to ensure cell transfection (e.g., in the cellular carrier), alter the biodistribution of the signal-sensor polynucleotide, primary construct, or mmRNA (e.g., by targeting the cell carrier to specific tissues or cell types), and/or increase the translation of encoded oncology-related protein.

[00451] A variety of methods are known in the art and suitable for introduction of nucleic acid into a cell, including viral and non-viral mediated techniques. Examples of typical non-viral mediated techniques include, but are not limited to, electroporation, calcium phosphate mediated transfer, nucleofection, sonoporation, heat shock, magnetofection, liposome mediated transfer, microinjection, microprojectile mediated transfer (nanoparticles), cationic polymer mediated transfer (DEAE-dextran, polyethylenimine, polyethylene glycol (PEG) and the like) or cell fusion.

[00452] The technique of sonoporation, or cellular sonication, is the use of sound (e.g., ultrasonic frequencies) for modifying the permeability of the cell plasma membrane. Sonoporation methods are known to those in the art and are used to deliver nucleic acids *in vivo* (Yoon and Park, Expert Opin Drug Deliv. 2010 7:321-330; Postema and Gilja, Curr Pharm Biotechnol. 2007 8:355-361; Newman and Bettinger, Gene Ther. 2007 14:465-475; all herein incorporated by reference in their entirety). Sonoporation methods are known in the art and are also taught for example as it relates to bacteria in US Patent Publication 20100196983 and as it relates to other cell types in, for example, US Patent Publication 20100009424, each of which are incorporated herein by reference in their entirety.

[00453] Electroporation techniques are also well known in the art and are used to deliver nucleic acids *in vivo* and clinically (Andre et al., Curr Gene Ther. 2010 10:267-280; Chiarella et al, Curr Gene Ther. 2010 10:281-286; Hojman, Curr Gene Ther. 2010

10:128-138; all herein incorporated by reference in their entirety). In one embodiment, signal-sensor polynucleotides, primary constructs or mmRNA may be delivered by electroporation as described in Example 12.

Hyaluronidase

[00454] The intramuscular or subcutaneous localized injection of signal-sensor polynucleotide, primary construct, or mmRNA of the invention can include hyaluronidase, which catalyzes the hydrolysis of hyaluronan. By catalyzing the hydrolysis of hyaluronan, a constituent of the interstitial barrier, hyaluronidase lowers the viscosity of hyaluronan, thereby increasing tissue permeability (Frost, Expert Opin. Drug Deliv. (2007) 4:427-440; herein incorporated by reference in its entirety). It is useful to speed their dispersion and systemic distribution of encoded proteins produced by transfected cells. Alternatively, the hyaluronidase can be used to increase the number of cells exposed to a signal-sensor polynucleotide, primary construct, or mmRNA of the invention administered intramuscularly or subcutaneously.

Nanoparticle Mimics

[00455] The signal-sensor polynucleotide, primary construct or mmRNA of the invention may be encapsulated within and/or absorbed to a nanoparticle mimic. A nanoparticle mimic can mimic the delivery function organisms or particles such as, but not limited to, pathogens, viruses, bacteria, fungus, parasites, prions and cells. As a non-limiting example the signal-sensor polynucleotide, primary construct or mmRNA of the invention may be encapsulated in a non-viron particle which can mimic the delivery function of a virus (see International Pub. No. WO20 12006376 herein incorporated by reference in its entirety).

Nanotubes

[00456] The signal-sensor polynucleotides, primary constructs or mmRNA of the invention can be attached or otherwise bound to at least one nanotube such as, but not limited to, rosette nanotubes, rosette nanotubes having twin bases with a linker, carbon nanotubes and/or single-walled carbon nanotubes, The signal-sensor polynucleotides, primary constructs or mmRNA may be bound to the nanotubes through forces such as, but not limited to, steric, ionic, covalent and/or other forces.

[00457] In one embodiment, the nanotube can release one or more signal-sensor polynucleotides, primary constructs or mmRNA into cells. The size and/or the surface structure of at least one nanotube may be altered so as to govern the interaction of the nanotubes within the body and/or to attach or bind to the signal-sensor polynucleotides, primary constructs or mmRNA disclosed herein. In one embodiment, the building block and/or the functional groups attached to the building block of the at least one nanotube may be altered to adjust the dimensions and/or properties of the nanotube. As a non-limiting example, the length of the nanotubes may be altered to hinder the nanotubes from passing through the holes in the walls of normal blood vessels but still small enough to pass through the larger holes in the blood vessels of tumor tissue.

[00458] In one embodiment, at least one nanotube may also be coated with delivery enhancing compounds including polymers, such as, but not limited to, polyethylene glycol. In another embodiment, at least one nanotube and/or the signal-sensor polynucleotides, primary constructs or mmRNA may be mixed with pharmaceutically acceptable excipients and/or delivery vehicles.

[00459] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA are attached and/or otherwise bound to at least one rosette nanotube. The rosette nanotubes may be formed by a process known in the art and/or by the process described in International Publication No. WO2012094304, herein incorporated by reference in its entirety. At least one signal-sensor polynucleotide, primary construct and/or mmRNA may be attached and/or otherwise bound to at least one rosette nanotube by a process as described in International Publication No. WO2012094304, herein incorporated by reference in its entirety, where rosette nanotubes or modules forming rosette nanotubes are mixed in aqueous media with at least one signal-sensor polynucleotide, primary construct and/or mmRNA under conditions which may cause at least one signal-sensor polynucleotide, primary construct or mmRNA to attach or otherwise bind to the rosette nanotubes.

Conjugates

[00460] The signal-sensor polynucleotides, primary constructs, and mmRNA of the invention include conjugates, such as a polynucleotide, primary construct, or mmRNA covalently linked to a carrier or targeting group, or including two encoding regions that

together produce a fusion protein (e.g., bearing a targeting group and therapeutic protein or peptide).

[00461] The conjugates of the invention include a naturally occurring substance, such as a protein (e.g., human serum albumin (HSA), low-density lipoprotein (LDL), high-density lipoprotein (HDL), or globulin); an carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid); or a lipid. The ligand may also be a recombinant or synthetic molecule, such as a synthetic polymer, e.g., a synthetic polyamino acid, an oligonucleotide (e.g. an aptamer). 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.

[00462] Representative U.S. patents that teach the preparation of polynucleotide conjugates, particularly to RNA, include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,1 18,802; 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; 5,1 12,963; 5,214,136; 5,082,830; 5,1 12,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; each of which is herein incorporated by reference in their entirety.

[00463] In one embodiment, the conjugate of the present invention may function as a carrier for the signal-sensor mmRNA of the present invention. The conjugate may comprise a cationic polymer such as, but not limited to, polyamine, polylysine,

polyalkylenimine, and polyethylenimine which may be grafted to with poly(ethylene glycol). As a non-limiting example, the conjugate may be similar to the polymeric conjugate and the method of synthesizing the polymeric conjugate described in U.S. Pat. No. 6,586,524 herein incorporated by reference in its entirety.

[00464] The conjugates can also include targeting groups, e.g., a cell or tissue targeting agent, e.g., a lectin, glycoprotein, lipid or protein, e.g., an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, biotin, an RGD peptide, an RGD peptide mimetic or an aptamer.

[00465] Targeting groups 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 cancer cell, endothelial cell, or bone cell. Targeting groups may 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, multivalent fucose, or aptamers. The ligand can be, for example, a lipopolysaccharide, or an activator of p38 MAP kinase.

[00466] The targeting group can be any ligand that is capable of targeting a specific receptor. Examples include, without limitation, folate, GalNAc, galactose, mannose, mannose-6P, apatamers, integrin receptor ligands, chemokine receptor ligands, transferrin, biotin, serotonin receptor ligands, PSMA, endothelin, GCPII, somatostatin, LDL, and HDL ligands. In particular embodiments, the targeting group is an aptamer. The aptamer can be unmodified or have any combination of modifications disclosed herein.

[00467] In one embodiment, pharmaceutical compositions of the present invention may include chemical modifications such as, but not limited to, modifications similar to locked nucleic acids.

[00468] Representative U.S. Patents that teach the preparation of locked nucleic acid (LNA) such as those from Santaris, include, but are not limited to, the following: U.S. Pat. Nos. 6,268,490; 6,670,461; 6,794,499; 6,998,484; 7,053,207; 7,084,125; and 7,399,845, each of which is herein incorporated by reference in its entirety.

[00469] Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found, for example, in Nielsen *et al.,* Science, 1991, 254, 1497-1500.

[00470] Some embodiments featured in the invention include signal-sensor polynucleotides, primary constructs or mmRNA with phosphorothioate backbones and oligonucleosides with other modified backbones, and in particular —CH_2—NH—CH_2—, —CH_2~N(CH_3)~0~CH_2~[known as a methylene (methylimino) or MMI backbone], —CH_2-0-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(0)$_2$—O—CH_2—] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. In some embodiments, the polynucletotides featured herein have morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

[00471] Modifications at the 2' position may also aid in delivery. Preferably, modifications at the 2' position are not located in a polypeptide-coding sequence, i.e., not in a translatable region. Modifications at the *2'* position may be located in a 5'UTR, a 3'UTR and/or a tailing region. Modifications at the *2'* position can include one of the following at the 2' position: H (i.e., 2'-deoxy); F; 0-, S-, or N-alkyl; 0-, S-, or N-alkenyl; 0-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted Ci to Cio alkyl or C_2 to Cio alkenyl and alkynyl. Exemplary suitable modifications include 0[(CH_2)$_n$O]$_m$$CH_3$, 0($CH_2$)$_n$$OCH_3$, 0($CH_2$)$_n$$NH_2$, 0($CH_2$)$_n$$CH_3$, 0($CH_2$)$_n$$ONH_2$, and 0($CH_2$)$_n$ON[($CH_2$)$_n$$CH_3$)]$_2$, where n and m are from 1 to about 10. In other embodiments, the signal-sensor polynucleotides, primary constructs or mmRNA include one of the following at the 2' position: Ci to Cio lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, CI, Br, CN, CF_3, OCF_3, $SOCH_3$, S0$_2$$CH_3$, ON0$_2$, N0$_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, or a group for improving the pharmacodynamic properties, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-0—CH_2CH_2OCH3, also known as 2'-0-(2-methoxy ethyl) 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 $0(CH_2)_20N(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-0-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.,* 2'-0-CH_2~0-CH_2~$N(CH_2)_2$, also described in examples herein below. Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions, 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. signal-sensor polynucleotides of the invention may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,1 18,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,81 1; 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 and each of which is herein incorporated by reference.

[00472] In still other embodiments, the signal-sensor polynucleotide, primary construct, or mmRNA is covalently conjugated to a cell penetrating polypeptide. The cell-penetrating peptide may also include a signal peptide sequence. The conjugates of the invention can be designed to have increased stability; increased cell transfection; and/or altered the biodistribution (e.g., targeted to specific tissues or cell types).

Self-Assembled Nucleic Acid Nanoparticles

[00473] Self-assembled nanoparticles have a well-defined size which may be precisely controlled as the nucleic acid strands may be easily reprogrammable. For example, the optimal particle size for a cancer-targeting nanodelivery carrier is 20-100 nm as a diameter greater than 20 nm avoids renal clearance and enhances delivery to certain

tumors through enhanced permeability and retention effect. Using self-assembled nucleic acid nanoparticles a single uniform population in size and shape having a precisely controlled spatial orientation and density of cancer-targeting ligands for enhanced delivery. As a non-limiting example, oligonucleotide nanoparticles were prepared using programmable self-assembly of short DNA fragments and therapeutic siRNAs. These nanoparticles are molecularly identical with controllable particle size and target ligand location and density. The DNA fragments and siRNAs self-assembled into a one-step reaction to generate DNA/siRNA tetrahedral nanoparticles for targeted *in vivo* delivery. (Lee et al, Nature Nanotechnology 2012 7:389-393).

Excipients

[00474] Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's *The Science and Practice of Pharmacy,* 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

[00475] In some embodiments, a pharmaceutically acceptable excipient is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient is approved for use in humans and for veterinary use. In some embodiments, an excipient is approved by United States Food and Drug Administration. In some embodiments, an excipient is pharmaceutical grade. In some embodiments, an excipient meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[00476] Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients may optionally be included in pharmaceutical compositions.

[00477] Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, *etc.*, and/or combinations thereof.

[00478] Exemplary granulating and/or dispersing agents include, but are not limited to, potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (VEEGUM®), sodium lauryl sulfate, quaternary ammonium compounds, *etc.*, and/or combinations thereof.

[00479] Exemplary surface active agents and/or emulsifiers include, but are not limited to, natural emulsifiers (e.g. acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (e.g. bentonite [aluminum silicate] and VEEGUM® [magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (e.g. stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (e.g. carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (e.g. carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty

acid esters (e.g. polyoxyethylene sorbitan monolaurate [TWEEN®20], polyoxy ethylene sorbitan [TWEENn®60], polyoxyethylene sorbitan monooleate [TWEEN®80], sorbitan monopalmitate [SPAN®40], sorbitan monostearate [Span®60], sorbitan tristearate [Span®65], glyceryl monooleate, sorbitan monooleate [SPAN®80]), polyoxyethylene esters (e.g. polyoxyethylene monostearate [MYRJ®45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and SOLUTOL®), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g. CREMOPHOR®), polyoxyethylene ethers, (e.g. polyoxyethylene lauryl ether [BRIJ®30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, PLUORINC®F 68, POLOXAMER®188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, etc. and/or combinations thereof.

[00480] Exemplary binding agents include, but are not limited to, starch *(e.g.* cornstarch and starch paste); gelatin; sugars *(e.g.* sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol,); natural and synthetic gums *(e.g.* acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate (VEEGUM®), and larch arabogalactan); alginates; polyethylene oxide; polyethylene glycol; inorganic calcium salts; silicic acid; polymethacrylates; waxes; water; alcohol; *etc.;* and combinations thereof.

[00481] Exemplary preservatives may include, but are not limited to, antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and/or other preservatives. Exemplary antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and/or sodium sulfite. Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium

edetate, tartaric acid, and/or trisodium edetate. Exemplary antimicrobial preservatives include, but are not limited to, benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and/or thimerosal. Exemplary antifungal preservatives include, but are not limited to, butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and/or sorbic acid. Exemplary alcohol preservatives include, but are not limited to, ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and/or phenylethyl alcohol. Exemplary acidic preservatives include, but are not limited to, vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and/or phytic acid. Other preservatives include, but are not limited to, tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisol (BHA), butylated hydroxytoluened (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, GLYDANT PLUS®, PHENONIP®, methylparaben, GERMALL®115, GERMABEN®II, NEOLONE™, KATHON™, and/or EUXYL®.

[00482] Exemplary buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, *etc.*, and/or combinations thereof.

[00483] Exemplary lubricating agents include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behanate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, *etc.,* and combinations thereof.

[00484] Exemplary oils include, but are not limited to, almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mink, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and/or combinations thereof.

[00485] Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and/or perfuming agents can be present in the composition, according to the judgment of the formulator.

Delivery

[00486] The present disclosure encompasses the delivery of signal-sensor polynucleotides, primary constructs or mmRNA for any of therapeutic, pharmaceutical, diagnostic or imaging by any appropriate route taking into consideration likely advances in the sciences of drug delivery. Delivery may be naked or formulated.

Naked Delivery

[00487] The signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be delivered to a cell naked. As used herein in, "naked" refers to delivering signal-sensor polynucleotides, primary constructs or mmRNA free from agents which promote transfection. For example, the polynucleotides, primary constructs or mmRNA delivered to the cell may contain no modifications. The naked signal-sensor

polynucleotides, primary constructs or mmRNA may be delivered to the cell using routes of administration known in the art and described herein.

Formulated Delivery

[00488] The signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be formulated, using the methods described herein. The formulations may contain signal-sensor polynucleotides, primary constructs or mmRNA which may be modified and/or unmodified. The formulations may further include, but are not limited to, cell penetration agents, a pharmaceutically acceptable carrier, a delivery agent, a bioerodible or biocompatible polymer, a solvent, and a sustained-release delivery depot. The formulated signal-sensor polynucleotides, primary constructs or mmRNA may be delivered to the cell using routes of administration known in the art and described herein.

[00489] The compositions may also be formulated for direct delivery to an organ or tissue in any of several ways in the art including, but not limited to, direct soaking or bathing, via a catheter, by gels, powder, ointments, creams, gels, lotions, and/or drops, by using substrates such as fabric or biodegradable materials coated or impregnated with the compositions, and the like.

Administration

[00490] The signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited to enteral, gastroenteral, epidural, oral, transdermal, epidural (peridural), intracerebral (into the cerebrum), intracerebroventricular (into the cerebral ventricles), epicutaneous (application onto the skin), intradermal, (into the skin itself), subcutaneous (under the skin), nasal administration (through the nose), intravenous (into a vein), intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), intraosseous infusion (into the bone marrow), intrathecal (into the spinal canal), intraperitoneal, (infusion or injection into the peritoneum), intravesical infusion, intravitreal, (through the eye), intracavernous injection, (into the base of the penis), intravaginal administration, intrauterine, extra-amniotic administration, transdermal (diffusion through the intact skin for systemic distribution), transmucosal (diffusion through a mucous membrane), insufflation

(snorting), sublingual, sublabial, enema, eye drops (onto the conjunctiva), or in ear drops. In specific embodiments, compositions may be administered in a way which allows them cross the blood-brain barrier, vascular barrier, or other epithelial barrier. Non-limiting routes of administration for the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention are described below.

Parenteral and Injectible Administration

[00491] Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and/or perfuming agents. In certain embodiments for parenteral administration, compositions are mixed with solubilizing agents such as CREMOPHOR®, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

[00492] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables.

[00493] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00494] In order to prolong the effect of an active ingredient, it is often desirable to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

Rectal and VaginalAdministration

[00495] Compositions for rectal or vaginal administration are typically suppositories which can be prepared by mixing compositions with suitable non-irritating excipients such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active ingredient.

Oral Administration

[00496] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, an active ingredient is mixed with at least one inert, pharmaceutically acceptable excipient such as sodium citrate or dicalcium phosphate and/or fillers or extenders *{e.g.* starches, lactose, sucrose, glucose, mannitol, and silicic acid), binders *{e.g.* carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia), humectants *{e.g.* glycerol), disintegrating agents *{e.g.* agar, calcium carbonate, potato or tapioca starch, alginic acid, certain

silicates, and sodium carbonate), solution retarding agents *(e.g.* paraffin), absorption accelerators *(e.g.* quaternary ammonium compounds), wetting agents *(e.g.* cetyl alcohol and glycerol monostearate), absorbents *(e.g.* kaolin and bentonite clay), and lubricants *(e.g.* talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate), and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may comprise buffering agents.

Topical or Transdermal Administration

[00497] As described herein, compositions containing the signal-sensor polynucleotides, primary constructs or mmRNA of the invention may be formulated for administration topically. The skin may be an ideal target site for delivery as it is readily accessible. Gene expression may be restricted not only to the skin, potentially avoiding nonspecific toxicity, but also to specific layers and cell types within the skin.

[00498] The site of cutaneous expression of the delivered compositions will depend on the route of nucleic acid delivery. Three routes are commonly considered to deliver signal-sensor polynucleotides, primary constructs or mmRNA to the skin: (i) topical application (e.g. for local/regional treatment and/or oncology-related applications); (ii) intradermal injection (e.g. for local/regional treatment and/or oncology-related applications); and (iii) systemic delivery (e.g. for treatment of dermatologic diseases that affect both cutaneous and extracutaneous regions). Signal-sensor polynucleotides, primary constructs or mmRNA can be delivered to the skin by several different approaches known in the art. Most topical delivery approaches have been shown to work for delivery of DNA, such as but not limited to, topical application of non-cationic liposome-DNA complex, cationic liposome-DNA complex, particle-mediated (gene gun), puncture-mediated gene transfections, and viral delivery approaches. After delivery of the nucleic acid, gene products have been detected in a number of different skin cell types, including, but not limited to, basal keratinocytes, sebaceous gland cells, dermal fibroblasts and dermal macrophages.

[00499] In one embodiment, the invention provides for a variety of dressings (e.g., wound dressings) or bandages (e.g., adhesive bandages) for conveniently and/or effectively carrying out methods of the present invention. Typically dressing or bandages may comprise sufficient amounts of pharmaceutical compositions and/or signal-sensor

polynucleotides, primary constructs or mmRNA described herein to allow a user to perform multiple treatments of a subject(s).

[00500] In one embodiment, the invention provides for the signal-sensor polynucleotides, primary constructs or mmRNA compositions to be delivered in more than one injection.

[00501] In one embodiment, before topical and/or transdermal administration at least one area of tissue, such as skin, may be subjected to a device and/or solution which may increase permeability. In one embodiment, the tissue may be subjected to an abrasion device to increase the permeability of the skin (see U.S. Patent Publication No. 20080275468, herein incorporated by reference in its entirety). In another embodiment, the tissue may be subjected to an ultrasound enhancement device. An ultrasound enhancement device may include, but is not limited to, the devices described in U.S. Publication No. 20040236268 and U.S. Patent Nos. 6,491,657 and 6,234,990; herein incorporated by reference in their entireties. Methods of enhancing the permeability of tissue are described in U.S. Publication Nos. 20040171980 and 20040236268 and U.S. Pat. No. 6,190,315; herein incorporated by reference in their entireties.

[00502] In one embodiment, a device may be used to increase permeability of tissue before delivering formulations of modified mRNA described herein. The permeability of skin may be measured by methods known in the art and/or described in U.S. Patent No. 6,190,315, herein incorporated by reference in its entirety. As a non-limiting example, a modified mRNA formulation may be delivered by the drug delivery methods described in U.S. Patent No. 6,190,315, herein incorporated by reference in its entirety.

[00503] In another non-limiting example tissue may be treated with a eutectic mixture of local anesthetics (EMLA) cream before, during and/or after the tissue may be subjected to a device which may increase permeability. Katz et al. (Anesth Analg (2004); 98:371-76; herein incorporated by reference in its entirety) showed that using the EMLA cream in combination with a low energy, an onset of superficial cutaneous analgesia was seen as fast as 5 minutes after a pretreatment with a low energy ultrasound.

[00504] In one embodiment, enhancers may be applied to the tissue before, during, and/or after the tissue has been treated to increase permeability. Enhancers include, but are not limited to, transport enhancers, physical enhancers, and cavitation enhancers.

Non-limiting examples of enhancers are described in U.S. Patent No. 6,190,315, herein incorporated by reference in its entirety.

[00505] In one embodiment, a device may be used to increase permeability of tissue before delivering formulations of modified mRNA described herein, which may further contain a substance that invokes an immune response. In another non-limiting example, a formulation containing a substance to invoke an immune response may be delivered by the methods described in U.S. Publication Nos. 20040171980 and 20040236268; herein incorporated by reference in their entireties.

[00506] Dosage forms for topical and/or transdermal administration of a composition may include ointments, pastes, creams, lotions, gels, foams, powders, solutions, sprays, inhalants and/or patches. Generally, an active ingredient is admixed under sterile conditions with a pharmaceutically acceptable excipient and/or any needed preservatives and/or buffers as may be required. Additionally, the present invention contemplates the use of transdermal patches, which often have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms may be prepared, for example, by dissolving and/or dispensing the compound in the proper medium. Alternatively or additionally, rate may be controlled by either providing a rate controlling membrane and/or by dispersing the compound in a polymer matrix and/or gel.

[00507] Formulations suitable for topical administration include, but are not limited to, liquid and/or semi liquid preparations such as liniments, lotions, oil in water and/or water in oil emulsions such as creams, ointments and/or pastes, and/or solutions and/or suspensions.

[00508] Topically-administrable formulations may, for example, comprise from about 0.1% to about 10% (w/w) active ingredient, although the concentration of active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

Penetration Enhancers

[00509] In one embodiment, the signal-sensor polynucleotides, primary construct and mmRNA of present invention may use various penetration enhancers to deliver the signal-sensor polynucleotides, primary construct and mmRNA to at least one area

associated with one or more hyperproliferative diseases, disorders or conditions. 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 may 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.

[00510] Penetration enhancers may be classified as belonging to one of five broad categories, i.e., 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 the above mentioned classes of penetration enhancers are described below in greater detail. Combinations of penetration enhancer may also be encompassed by the scope of the present invention, for example, fatty acids/salts in combination with bile acids/salts. Other non-limiting examples of combinations of penetration enhancers include the combination of sodium salt of lauric acid, capric acid and UDCA.

Surfactants

[00511] In connection with the present invention, surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of the signal-sensor polynucleotides, primary constructs and mmRNA through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92); and perfluorochemical emulsions, such as FC-43 (Takahashi et al., J. Pharm. Pharmacol., 1988, 40, 252).

Fatty acids

[00512] Various fatty acids and their derivatives which act as penetration enhancers include, but are not limited to, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, l-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, **Ci**

- **Cio** alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al, Critical Reviews in Therapeutic Drug Carryier Systems, 1991, p.92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri et al, J. Pharm. Pharmacol, 1992, 44, 651-654).

Bile salts

[00513] The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman et al. Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. The bile salts of the invention include, but are not limited to, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto et al, J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita et al, J. Pharm. Sci., 1990, 79, 579-583).

Chelating Agents

[00514] Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of signal-sensor polynucleotides, primary construct and mmRNA through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage

of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315-339). Chelating agents of the invention include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 andN-amino acyl derivatives of beta-diketones (enamines)(Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur et al, J. Control ReL, 1990, 14, 43-51).

Non-chelating non-surfactants

[00515] As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of signal-sensor polynucleotides, primary construct and mmRNA through the alimentary mucosa (Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers include, but are not limited to, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., J. Pharm. Pharmacol, 1987, 39, 621-626).

[00516] Agents that enhance uptake of signal-sensor polynucleotides, primary construct and mmRNA at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi et al, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo et al, PCT Application WO 97/30731), are also known to enhance the cellular uptake of signal-sensor polynucleotides, primary construct and mmRNA.

[00517] Other agents may be utilized to enhance the penetration of the administered signal-sensor polynucleotides, primary construct and mmRNA, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

Depot Administration

[00518] As described herein, in some embodiments, the composition is formulated in depots for extended release. Generally, a specific organ or tissue (a "target tissue") is targeted for administration.

[00519] In some aspects of the invention, the signal-sensor polynucleotides, primary constructs or mmRNA are spatially retained within or proximal to a target tissue. Provided are method of providing a composition to a target tissue of a mammalian subject by contacting the target tissue (which contains one or more target cells) with the composition under conditions such that the composition, in particular the nucleic acid component(s) of the composition, is substantially retained in the target tissue, meaning that at least 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the composition is retained in the target tissue. Advantageously, retention is determined by measuring the amount of the nucleic acid present in the composition that enters one or more target cells. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the nucleic acids administered to the subject are present intracellularly at a period of time following administration. For example, intramuscular injection to a mammalian subject is performed using an aqueous composition containing a ribonucleic acid and a transfection reagent, and retention of the composition is determined by measuring the amount of the ribonucleic acid present in the muscle cells.

[00520] Aspects of the invention are directed to methods of providing a composition to a target tissue of a mammalian subject, by contacting the target tissue (containing one or more target cells) with the composition under conditions such that the composition is substantially retained in the target tissue. The composition contains an effective amount of a signal-sensor polynucleotides, primary constructs or mmRNA such that the polypeptide of interest is produced in at least one target cell. The compositions generally contain a cell penetration agent, although "naked" nucleic acid (such as nucleic acids without a cell penetration agent or other agent) is also contemplated, and a pharmaceutically acceptable carrier.

[00521] In some circumstances, the amount of an oncology-related protein produced by cells in a tissue is desirably increased. Preferably, this increase in oncology-related

protein production is spatially restricted to cells within the target tissue. Thus, provided are methods of increasing production of an oncology-related protein of interest in a tissue of a mammalian subject. A composition is provided that contains signal-sensor polynucleotides, primary constructs or mmRNA characterized in that a unit quantity of composition has been determined to produce the polypeptide of interest in a substantial percentage of cells contained within a predetermined volume of the target tissue.

[00522] In some embodiments, the composition includes a plurality of different signal-sensor polynucleotides, primary constructs or mmRNA, where one or more than one of the signal-sensor polynucleotides, primary constructs or mmRNA encodes an oncology-related polypeptide of interest. Optionally, the composition also contains a cell penetration agent to assist in the intracellular delivery of the composition. A determination is made of the dose of the composition required to produce the oncology-related polypeptide of interest in a substantial percentage of cells contained within the predetermined volume of the target tissue (generally, without inducing significant production of the oncology-related polypeptide of interest in tissue adjacent to the predetermined volume, or distally to the target tissue). Subsequent to this determination, the determined dose is introduced directly into the tissue of the mammalian subject.

[00523] In one embodiment, the invention provides for the signal-sensor polynucleotides, primary constructs or mmRNA to be delivered in more than one injection or by split dose injections.

[00524] In one embodiment, the invention may be retained near target tissue using a small disposable drug reservoir or patch pump. Non-limiting examples of patch pumps include those manufactured and/or sold by BD® (Franklin Lakes, NJ), Insulet Corporation (Bedford, MA), SteadyMed Therapeutics (San Francisco, CA), Medtronic (Minneapolis, MN), UniLife (York, PA), Valeritas (Bridgewater, NJ), and SpringLeaf Therapeutics (Boston, MA).

Pulmonary Administration

[00525] A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 nm to about 7 nm or from about 1 nm to

about 6 nm. Such compositions are suitably in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder and/or using a self propelling solvent/powder dispensing container such as a device comprising the active ingredient dissolved and/or suspended in a low-boiling propellant in a sealed container. Such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nm and at least 95% of the particles by number have a diameter less than 7 nm. Alternatively, at least 95% of the particles by weight have a diameter greater than 1 nm and at least 90% of the particles by number have a diameter less than 6 nm. Dry powder compositions may include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

[00526] Low boiling propellants generally include liquid propellants having a boiling point of below 65 °F at atmospheric pressure. Generally the propellant may constitute 50% to 99.9% (w/w) of the composition, and active ingredient may constitute 0.1% to 20% (w/w) of the composition. A propellant may further comprise additional ingredients such as a liquid non-ionic and/or solid anionic surfactant and/or a solid diluent (which may have a particle size of the same order as particles comprising the active ingredient).

[00527] Pharmaceutical compositions formulated for pulmonary delivery may provide an active ingredient in the form of droplets of a solution and/or suspension. Such formulations may be prepared, packaged, and/or sold as aqueous and/or dilute alcoholic solutions and/or suspensions, optionally sterile, comprising active ingredient, and may conveniently be administered using any nebulization and/or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, and/or a preservative such as methylhydroxybenzoate. Droplets provided by this route of administration may have an average diameter in the range from about 0.1 nm to about 200 nm.

Intranasal, nasal and buccal Administration

[00528] Formulations described herein as being useful for pulmonary delivery are useful for intranasal delivery of a pharmaceutical composition. Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient

and having an average particle from about 0.2 μιη to 500 μιη. Such a formulation is administered in the manner in which snuff is taken, *i.e.* by rapid inhalation through the nasal passage from a container of the powder held close to the nose.

[00529] Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of active ingredient, and may comprise one or more of the additional ingredients described herein. A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets and/or lozenges made using conventional methods, and may, for example, 0.1% to 20% (w/w) active ingredient, the balance comprising an orally dissolvable and/or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder and/or an aerosolized and/or atomized solution and/or suspension comprising active ingredient. Such powdered, aerosolized, and/or aerosolized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 nm to about 200 nm, and may further comprise one or more of any additional ingredients described herein.

Ophthalmic Administration

[00530] A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1/1.0% (w/w) solution and/or suspension of the active ingredient in an aqueous or oily liquid excipient. Such drops may further comprise buffering agents, salts, and/or one or more other of any additional ingredients described herein. Other ophthalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form and/or in a liposomal preparation. Ear drops and/or eye drops are contemplated as being within the scope of this invention.

Payload Administration: Detectable Agents and Therapeutic Agents

[00531] The signal-sensor polynucleotides, primary constructs or mmRNA described herein can be used in a number of different scenarios in which delivery of a substance

(the "payload") to a biological target is desired, for example delivery of detectable substances for detection of the target, or delivery of a therapeutic agent. Detection methods can include, but are not limited to, both imaging *in vitro* and *in vivo* imaging methods, *e.g.*, immunohistochemistry, bioluminescence imaging (BLI), Magnetic Resonance Imaging (MRI), positron emission tomography (PET), electron microscopy, X-ray computed tomography, Raman imaging, optical coherence tomography, absorption imaging, thermal imaging, fluorescence reflectance imaging, fluorescence microscopy, fluorescence molecular tomographic imaging, nuclear magnetic resonance imaging, X-ray imaging, ultrasound imaging, photoacoustic imaging, lab assays, or in any situation where tagging/staining/imaging is required.

[00532] The signal-sensor polynucleotides, primary constructs or mmRNA can be designed to include both a linker and a payload in any useful orientation. For example, a linker having two ends is used to attach one end to the payload and the other end to the nucleobase, such as at the C-7 or C-8 positions of the deaza-adenosine or deaza-guanosine or to the N-3 or C-5 positions of cytosine or uracil. The signal-sensor polynucleotide of the invention can include more than one payload (e.g., a label and a transcription inhibitor), as well as a cleavable linker. In one embodiment, the modified nucleotide is a modified 7-deaza-adenosine triphosphate, where one end of a cleavable linker is attached to the C7 position of 7-deaza-adenine, the other end of the linker is attached to an inhibitor (e.g., to the C5 position of the nucleobase on a cytidine), and a label (e.g., Cy5) is attached to the center of the linker (see, e.g., compound 1 of A*pCp C5 Parg Capless in Fig. 5 and columns 9 and 10 of U.S. Pat. No. 7,994,304, incorporated herein by reference). Upon incorporation of the modified 7-deaza-adenosine triphosphate to an encoding region, the resulting signal-sensor polynucleotide having a cleavable linker attached to a label and an inhibitor (e.g., a polymerase inhibitor). Upon cleavage of the linker (e.g., with reductive conditions to reduce a linker having a cleavable disulfide moiety), the label and inhibitor are released. Additional linkers and payloads (e.g., therapeutic agents, detectable labels, and cell penetrating payloads) are described herein.

[00533] For example, the signal-sensor polynucleotides, primary constructs or mmRNA described herein can be used in reprogramming induced pluripotent stem cells (iPS cells),

which can directly track cells that are transfected compared to total cells in the cluster. In another example, a drug that may be attached to the signal-sensor polynucleotides, primary constructs or mmRNA via a linker and may be fluorescently labeled can be used to track the drug *in vivo, e.g.* intracellularly. Other examples include, but are not limited to, the use of signal-sensor polynucleotides, primary constructs or mmRNA in reversible drug delivery into cells.

[00534] The signal-sensor polynucleotides, primary constructs or mmRNA described herein can be used in intracellular targeting of a payload, *e.g.,* detectable or therapeutic agent, to specific organelle. Exemplary intracellular targets can include, but are not limited to, the nuclear localization for advanced mRNA processing, or a nuclear localization sequence (NLS) linked to the mRNA containing an inhibitor.

[00535] In addition, the signal-sensor polynucleotides, primary constructs or mmRNA described herein can be used to deliver therapeutic agents to cells or tissues, *e.g.,* in living animals. For example, the signal-sensor polynucleotides, primary constructs or mmRNA described herein can be used to deliver highly polar chemotherapeutics agents to kill cancer cells. The signal-sensor polynucleotides, primary constructs or mmRNA attached to the therapeutic agent through a linker can facilitate member permeation allowing the therapeutic agent to travel into a cell to reach an intracellular target.

[00536] In another example, the signal-sensor polynucleotides, primary constructs or mmRNA can be attached to the polynucleotides, primary constructs or mmRNA a viral inhibitory peptide (VIP) through a cleavable linker. The cleavable linker can release the VIP and dye into the cell. In another example, the signal-sensor polynucleotides, primary constructs or mmRNA can be attached through the linker to an ADP-ribosylate, which is responsible for the actions of some bacterial toxins, such as cholera toxin, diphtheria toxin, and pertussis toxin. These toxin proteins are ADP-ribosyltransferases that modify target proteins in human cells. For example, cholera toxin ADP-ribosylates G proteins modifies human cells by causing massive fluid secretion from the lining of the small intestine, which results in life-threatening diarrhea.

[00537] In some embodiments, the payload may be a therapeutic agent such as a cytotoxin, radioactive ion, chemotherapeutic, or other therapeutic agent. A cytotoxin or cytotoxic agent includes any agent that may be detrimental to cells. Examples include,

but are not limited to, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxyanthracmedione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, *e.g.*, maytansinol (see U.S. Pat. No. 5,208,020 incorporated herein in its entirety), rachelmycin (CC-1065, see U.S. Pat. Nos. 5,475,092, 5,585,499, and 5,846,545, all of which are incorporated herein by reference), and analogs or homologs thereof. Radioactive ions include, but are not limited to iodine *{e.g.*, iodine 125 or iodine 131), strontium 89, phosphorous, palladium, cesium, iridium, phosphate, cobalt, yttrium 90, samarium 153, and praseodymium. Other therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thiotepa chlorambucil, rachelmycin (CC-1065), melphalan, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine, vinblastine, taxol and maytansinoids).

[00538] In some embodiments, the payload may be a detectable agent, such as various organic small molecules, inorganic compounds, nanoparticles, enzymes or enzyme substrates, fluorescent materials, luminescent materials (e.g., luminol), bioluminescent materials (e.g., luciferase, luciferin, and aequorin), chemiluminescent materials, radioactive materials (e.g., ^{18}F, ^{67}Ga, ^{81m}Kr, ^{82}Rb, ^{111}In, ^{123}I, ^{133}Xe, $^{201}T1$, $^{125}1$, ^{35}S, ^{14}C, ^{3}H, or ^{99m}Tc (e.g., as pertechnetate (technetate(VII), $Tc0_4^-$)), and contrast agents (e.g., gold *{e.g.*, gold nanoparticles), gadolinium (e.g., chelated Gd), iron oxides (e.g., superparamagnetic iron oxide (SPIO), monocrystalline iron oxide nanoparticles (MIONs), and ultrasmall superparamagnetic iron oxide (USPIO)), manganese chelates (e.g., Mn-DPDP), barium sulfate, iodinated contrast media (iohexol), microbubbles, or perfluorocarbons). Such optically-detectable labels include for example, without limitation, 4-acetamido-4'-isothiocyanatostilbene-2,2'disulfonic acid; acridine and derivatives (e.g., acridine and acridine isothiocyanate); 5-(2'-

aminoethyl)aminonaphthalene-l -sulfonic acid (EDANS); 4-amino-N-[3-vinylsulfonyl)phenyl]naphthalimide-3 ,5 disulfonate; N-(4-anilino-l-naphthyl)maleimide; anthranilamide; BODIPY; Brilliant Yellow; coumarin and derivatives (e.g., coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120), and 7-amino-4-trifluoromethylcoumarin (Coumarin 151)); cyanine dyes; cyanosine; 4',6-diaminidino-2-phenylindole (DAPI); 5' 5''-dibromopyrogallol-sulfonaphthalein (Bromopyrogallol Red); 7-diethylamino-3 -(4'-isothiocyanatophenyl)-4-methylcoumarin; diethylenetriamine pentaacetate; 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid; 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; 5-[dimethylamino]-naphthalene-l-sulfonyl chloride (DNS, dansylchloride); 4-dimethylaminophenylazophenyl-4'-isothiocyanate (DABITC); eosin and derivatives (e.g., eosin and eosin isothiocyanate); erythrosin and derivatives (e.g., erythrosin B and erythrosin isothiocyanate); ethidium; fluorescein and derivatives (e.g., 5-carboxyfluorescein (FAM), 5-(4,6-dichlorotriazin-2-yl)amino fluorescein (DTAF), 2',7'-dimethoxy-4'5'-dichloro-6-carboxyfluorescein, fluorescein, fluorescein isothiocyanate, X-rhodamine-5-(and-6)-isothiocyanate (QFITC or XRITC), and fluorescamine); 2-[2-[3-[[l,3-dihydro-l,l-dimethyl-3-(3-sulfopropyl)-2H-benz[e]indol-2-ylidene]ethylidene]-2-[4-(ethoxycarbonyl)-l-piperazinyl]-l-cyclopenten-1 -yljethenyl]- 1,1-dimethyl-3-(3-sulforpropyl)- lH-benz[e]indolium hydroxide, inner salt, compound with n,n-diethylethanamine(l:l) (IR144); 5-chloro-2-[2-[3-[(5-chloro-3-ethyl-2(3H)-benzothiazol- ylidene)ethylidene]-2-(diphenylamino)-l-cyclopenten-l-yl]ethenyl]-3-ethyl benzothiazolium perchlorate (IR140); Malachite Green isothiocyanate; 4-methylumbelliferone orthocresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B-phycoerythrin; o-phthaldialdehyde; pyrene and derivatives(e.g., pyrene, pyrene butyrate, and succinimidyl 1-pyrene); butyrate quantum dots; Reactive Red 4 (CIBACRON™ Brilliant Red 3B-A); rhodamine and derivatives (e.g., 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride rhodamine (Rhod), rhodamine B, rhodamine 123, rhodamine X isothiocyanate, sulforhodamine B, sulforhodamine 101, sulfonyl chloride derivative of sulforhodamine 101 (Texas Red), N,N,N',N'tetramethyl-6-carboxyrhodamine (TAMRA) tetramethyl rhodamine, and tetramethyl rhodamine isothiocyanate (TRITC)); riboflavin; rosolic acid; terbium chelate derivatives; Cyanine-3 (Cy3); Cyanine-5 (Cy5); cyanine-5.5

(Cy5.5), Cyanine-7 (Cy7); IRD 700; IRD 800; Alexa 647; La Jolta Blue; phthalo cyanine; and naphthalo cyanine.

[00539] In some embodiments, the detectable agent may be a non-detectable pre-cursor that becomes detectable upon activation (e.g., fluorogenic tetrazine-fluorophore constructs (e.g., tetrazine-BODIPY FL, tetrazine-Oregon Green 488, or tetrazine-BODIPY TMR-X) or enzyme activatable fluorogenic agents (e.g., PROSENSE® (VisEn Medical))). In vitro assays in which the enzyme labeled compositions can be used include, but are not limited to, enzyme linked immunosorbent assays (ELISAs), immunoprecipitation assays, immunofluorescence, enzyme immunoassays (EIA), radioimmunoassays (RIA), and Western blot analysis. *Combinations*

[00540] The signal-sensor polynucleotides, primary constructs or mmRNA may be used in combination with one or more other therapeutic, prophylactic, diagnostic, or imaging agents. By "in combination with," it is not intended to imply that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the present disclosure. Compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In some embodiments, the present disclosure encompasses the delivery of pharmaceutical, prophylactic, diagnostic, or imaging compositions in combination with agents that may improve their bioavailability, reduce and/or modify their metabolism, inhibit their excretion, and/or modify their distribution within the body. As a non-limiting example, the signal-sensor nucleic acids or mmRNA may be used in combination with a pharmaceutical agent for the treatment of cancer or to control hyperproliferative cells. In U.S. Pat. No. 7,964,571, herein incorporated by reference in its entirety, a combination therapy for the treatment of solid primary or metastasized tumor is described using a pharmaceutical composition including a DNA plasmid encoding for interleukin-12 with a lipopolymer and also administering at least one anticancer agent or chemotherapeutic. Further, the signal-sensor nucleic acids and mmRNA of the present invention that encodes anti-proliferative molecules may be in a pharmaceutical composition with a lipopolymer (see e.g., U.S. Pub. No. 201 10218231, herein incorporated by reference in its entirety, claiming a

pharmaceutical composition comprising a DNA plasmid encoding an anti-proliferative molecule and a lipopolymer) which may be administered with at least one chemotherapeutic or anticancer agent.

Dosing

[00541] The present invention provides methods comprising administering modified mRNAs and their encoded proteins or complexes in accordance with the invention to a subject in need thereof. Nucleic acids, proteins or complexes, or pharmaceutical, imaging, diagnostic, or prophylactic compositions thereof, may be administered to a subject using any amount and any route of administration effective for preventing, treating, diagnosing, or imaging a disease, disorder, and/or condition *{e.g.*, a disease, disorder, and/or condition relating to working memory deficits). The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. Compositions in accordance with the invention are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present invention may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

[00542] In certain embodiments, compositions in accordance with the present invention may be administered at dosage levels sufficient to deliver from about 0.0001 mg/kg to about 100 mg/kg, from about 0.001 mg/kg to about 0.05 mg/kg, from about 0.005 mg/kg to about 0.05 mg/kg, from about 0.001 mg/kg to about 0.005 mg/kg, from about 0.05 mg/kg to about 0.5 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, from about 0.1 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 0.01

mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, or from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic, diagnostic, prophylactic, or imaging effect. The desired dosage may be delivered three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks. In certain embodiments, the desired dosage may be delivered using multiple administrations *(e.g.,* two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations).

[00543] According to the present invention, it has been discovered that administration of mmRNA in split-dose regimens produce higher levels of proteins in mammalian subjects. As used herein, a "split dose" is the division of single unit dose or total daily dose into two or more doses, e.g, two or more administrations of the single unit dose. As used herein, a "single unit dose" is a dose of any therapeutic administed in one dose/at one time/single route/single point of contact, i.e., single administration event. As used herein, a "total daily dose" is an amount given or prescribed in 24 hr period. It may be administered as a single unit dose. In one embodiment, the mmRNA of the present invention are administed to a subject in split doses. The mmRNA may be formulated in buffer only or in a formulation described herein.

Dosage Forms

[00544] A pharmaceutical composition described herein can be formulated into a dosage form described herein, such as a topical, intranasal, intratracheal, or injectable (e.g., intravenous, intraocular, intravitreal, intramuscular, intracardiac, intraperitoneal, subcutaneous).

Liquid dosage forms

[00545] Liquid dosage forms for parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art including, but not limited to, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn,

germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. In certain embodiments for parenteral administration, compositions may be mixed with solubilizing agents such as CREMOPHOR®, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

Injectable

[00546] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art and may include suitable dispersing agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed include, but are not limited to, are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables.

[00547] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00548] In order to prolong the effect of an active ingredient, it may be desirable to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the signal-sensor polynucleotide, primary construct or mmRNA then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered signal-sensor polynucleotide, primary construct or mmRNA may be accomplished by dissolving or suspending the signal-sensor polynucleotide, primary construct or mmRNA in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the signal-sensor polynucleotide,

primary construct or mmRNA in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of the signal-sensor polynucleotide, primary construct or mmRNA to polymer and the nature of the particular polymer employed, the rate of signal-sensor polynucleotide, primary construct or mmRNA release can be controlled. Examples of other biodegradable polymers include, but are not limited to, poly(orthoesters) and poly(anhydrides). Depot injectable formulations may be prepared by entrapping the signal-sensor polynucleotide, primary construct or mmRNA in liposomes or microemulsions which are compatible with body tissues.

Pulmonary

[00549] Formulations described herein as being useful for pulmonary delivery may also be use for intranasal delivery of a pharmaceutical composition. Another formulation suitable for intranasal administration may be a coarse powder comprising the active ingredient and having an average particle from about 0.2 μιη to 500 μιη. Such a formulation may be administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close to the nose.

[00550] Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of active ingredient, and may comprise one or more of the additional ingredients described herein. A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets and/or lozenges made using conventional methods, and may, for example, contain about 0.1% to 20% (w/w) active ingredient, where the balance may comprise an orally dissolvable and/or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder and/or an aerosolized and/or atomized solution and/or suspension comprising active ingredient. Such powdered, aerosolized, and/or aerosolized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 nm to about 200 nm, and may further comprise one or more of any additional ingredients described herein.

[00551] General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference).

Coatings or Shells

[00552] Solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally comprise opacifying agents and can be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

Properties of Pharmaceutical Compositions

[00553] The pharmaceutical compositions described herein can be characterized by one or more of bioavailability, therapeutic window and/or volume of distribution.

Bioavailability

[00554] The signal-sensor polynucleotides, primary constructs or mmRNA, when formulated into a composition with a delivery agent as described herein, can exhibit an increase in bioavailability as compared to a composition lacking a delivery agent as described herein. As used herein, the term "bioavailability" refers to the systemic availability of a given amount of signal-sensor polynucleotides, primary constructs or mmRNA administered to a mammal. Bioavailability can be assessed by measuring the area under the curve (AUC) or the maximum serum or plasma concentration (C_{max}) of the unchanged form of a compound following administration of the compound to a mammal. AUC is a determination of the area under the curve plotting the serum or plasma concentration of a compound along the ordinate (Y-axis) against time along the abscissa (X-axis). Generally, the AUC for a particular compound can be calculated using methods known to those of ordinary skill in the art and as described in G. S. Banker, Modern

Pharmaceutics, Drugs and the Pharmaceutical Sciences, v. 72, Marcel Dekker, New York, Inc., 1996, herein incorporated by reference.

[00555] The C_{max} value is the maximum concentration of the compound achieved in the serum or plasma of a mammal following administration of the compound to the mammal. The C_{max} value of a particular compound can be measured using methods known to those of ordinary skill in the art. The phrases "increasing bioavailability" or "improving the pharmacokinetics," as used herein mean that the systemic availability of a first signal-sensor polynucleotide, primary construct or mmRNA, measured as AUC, C_{max}, or $\mathbf{C_{min}}$ in a mammal is greater, when co-administered with a delivery agent as described herein, than when such co-administration does not take place. In some embodiments, the bioavailability of the signal-sensor polynucleotide, primary construct or mmRNA can increase by at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%>, at least about 55%, at least about 60%>, at least about 65%, at least about 70%>, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100%.

Therapeutic Window

[00556] The signal-sensor polynucleotides, primary constructs or mmRNA, when formulated into a composition with a delivery agent as described herein, can exhibit an increase in the therapeutic window of the administered signal-sensor polynucleotide, primary construct or mmRNA composition as compared to the therapeutic window of the administered signal-sensor polynucleotide, primary construct or mmRNA composition lacking a delivery agent as described herein. As used herein "therapeutic window" refers to the range of plasma concentrations, or the range of levels of therapeutically active substance at the site of action, with a high probability of eliciting a therapeutic effect. In some embodiments, the therapeutic window of the signal-sensor polynucleotide, primary construct or mmRNA when co-administered with a delivery agent as described herein can increase by at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at

least about 65%, at least about 70%>, at least about 75%, at least about 80%>, at least about 85%, at least about 90%, at least about 95%, or about 100%.

Volume of Distribution

[00557] The signal-sensor polynucleotides, primary constructs or mmRNA, when formulated into a composition with a delivery agent as described herein, can exhibit an improved volume of distribution ($V^{\wedge}_{st}$), e.g., reduced or targeted, relative to a composition lacking a delivery agent as described herein. The volume of distribution (Vdist) relates the amount of the drug in the body to the concentration of the drug in the blood or plasma. As used herein, the term "volume of distribution" refers to the fluid volume that would be required to contain the total amount of the drug in the body at the same concentration as in the blood or plasma: Vdist equals the amount of drug in the body/concentration of drug in blood or plasma. For example, for a 10 mg dose and a plasma concentration of 10 mg/L, the volume of distribution would be 1 liter. The volume of distribution reflects the extent to which the drug is present in the extravascular tissue. A large volume of distribution reflects the tendency of a compound to bind to the tissue components compared with plasma protein binding. In a clinical setting, V_{dist} can be used to determine a loading dose to achieve a steady state concentration. In some embodiments, the volume of distribution of the signal-sensor polynucleotide, primary construct or mmRNA when co-administered with a delivery agent as described herein can decrease at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%.

Biological Effect

[00558] In one embodiment, the biological effect of the signal-sensor modified mRNA delivered to the animals may be categorized by analyzing the protein expression in the animals. The protein expression may be determined from analyzing a biological sample collected from a mammal administered the signal-sensor modified mRNA of the present invention. In one embodiment, the expression protein encoded by the signal-sensor modified mRNA administered to the mammal of at least 50 pg/ml may be preferred. For example, a protein expression of 50-200 pg/ml for the protein encoded by the signal-

sensor modified mRNA delivered to the mammal may be seen as a therapeutically effective amount of protein in the mammal.

Detection of Modified Nucleic Acids by Mass Spectrometry

[00559] Mass spectrometry (MS) is an analytical technique that can provide structural and molecular mass/concentration information on molecules after their conversion to ions. The molecules are first ionized to acquire positive or negative charges and then they travel through the mass analyzer to arrive at different areas of the detector according to their mass/charge (m/z) ratio.

[00560] Mass spectrometry is performed using a mass spectrometer which includes an ion source for ionizing the fractionated sample and creating charged molecules for further analysis. For example ionization of the sample may be performed by electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), photoionization, electron ionization, fast atom bombardment (FAB)/liquid secondary ionization (LSIMS), matrix assisted laser desorption/ionization (MALDI), field ionization, field desorption, thermospray/plasmaspray ionization, and particle beam ionization. The skilled artisan will understand that the choice of ionization method can be determined based on the analyte to be measured, type of sample, the type of detector, the choice of positive versus negative mode, etc.

[00561] After the sample has been ionized, the positively charged or negatively charged ions thereby created may be analyzed to determine a mass-to-charge ratio (i.e., m/z). Suitable analyzers for determining mass-to-charge ratios include quadropole analyzers, ion traps analyzers, and time-of-flight analyzers. The ions may be detected using several detection modes. For example, selected ions may be detected (i.e., using a selective ion monitoring mode (SIM)), or alternatively, ions may be detected using a scanning mode, e.g., multiple reaction monitoring (MRM) or selected reaction monitoring (SRM).

[00562] Liquid chromatography-multiple reaction monitoring (LC-MS/MRM) coupled with stable isotope labeled dilution of peptide standards has been shown to be an effective method for protein verification (e.g., Keshishian et al., Mol Cell Proteomics 2009 8: 2339-2349; Kuhn et al, Clin Chem 2009 55:1 108-1 117; Lopez et al, Clin Chem 2010 56:281-290). Unlike untargeted mass spectrometry frequently used in biomarker discovery studies, targeted MS methods are peptide sequence-based modes of MS that

focus the full analytical capacity of the instrument on tens to hundreds of selected peptides in a complex mixture. By restricting detection and fragmentation to only those peptides derived from proteins of interest, sensitivity and reproducibility are improved dramatically compared to discovery-mode MS methods. This method of mass spectrometry-based multiple reaction monitoring (MRM) quantitation of proteins can dramatically impact the discovery and quantitation of biomarkers via rapid, targeted, multiplexed protein expression profiling of clinical samples.

[00563] In one embodiment, a biological sample which may contain at least one protein encoded by at least one modified mRNA of the present invention may be analyzed by the method of MRM-MS. The quantification of the biological sample may further include, but is not limited to, isotopically labeled peptides or proteins as internal standards.

[00564] According to the present invention, the biological sample, once obtained from the subject, may be subjected to enzyme digestion. As used herein, the term "digest" means to break apart into shorter peptides. As used herein, the phrase "treating a sample to digest proteins" means manipulating a sample in such a way as to break down proteins in a sample. These enzymes include, but are not limited to, trypsin, endoproteinase Glu-C and chymotrypsin. In one embodiment, a biological sample which may contain at least one protein encoded by at least one modified mRNA of the present invention may be digested using enzymes.

[00565] In one embodiment, a biological sample which may contain protein encoded by modified mRNA of the present invention may be analyzed for protein using electrospray ionization. Electrospray ionization (ESI) mass spectrometry (ESIMS) uses electrical energy to aid in the transfer of ions from the solution to the gaseous phase before they are analyzed by mass spectrometry. Samples may be analyzed using methods known in the art (e.g., Ho et al., Clin Biochem Rev. 2003 24(1):3-12). The ionic species contained in solution may be transferred into the gas phase by dispersing a fine spray of charge droplets, evaporating the solvent and ejecting the ions from the charged droplets to generate a mist of highly charged droplets. The mist of highly charged droplets may be analyzed using at least 1, at least 2, at least 3 or at least 4 mass analyzers such as, but not limited to, a quadropole mass analyzer. Further, the mass spectrometry method may include a purification step. As a non-limiting example, the first quadrapole may be set to

select a single m/z ratio so it may filter out other molecular ions having a different m/z ratio which may eliminate complicated and time-consuming sample purification procedures prior to MS analysis.

[00566] In one embodiment, a biological sample which may contain protein encoded by modified mRNA of the present invention may be analyzed for protein in a tandem ESIMS system (e.g., MS/MS). As non-limiting examples, the droplets may be analyzed using a product scan (or daughter scan) a precursor scan (parent scan) a neutral loss or a multiple reaction monitoring.

[00567] In one embodiment, a biological sample which may contain protein encoded by modified mRNA of the present invention may be analyzed using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MALDIMS). MALDI provides for the nondestructive vaporization and ionization of both large and small molecules, such as proteins. In MALDI analysis, the analyte is first co-crystallized with a large molar excess of a matrix compound, which may also include, but is not limited to, an ultraviolet absorbing weak organic acid. Non-limiting examples of matrices used in MALDI are a-cyano-4-hydroxycinnamic acid, 3,5-dimethoxy-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid. Laser radiation of the analyte-matrix mixture may result in the vaporization of the matrix and the analyte. The laser induced desorption provides high ion yields of the intact analyte and allows for measurement of compounds with high accuracy. Samples may be analyzed using methods known in the art (e.g., Lewis, Wei and Siuzdak, Encyclopedia of Analytical Chemistry 2000:5880-5894). As non-limiting examples, mass analyzers used in the MALDI analysis may include a linear time-of-flight (TOF), a TOF reflectron or a Fourier transform mass analyzer.

[00568] In one embodiment, the analyte-matrix mixture may be formed using the dried-droplet method. A biologic sample is mixed with a matrix to create a saturated matrix solution where the matrix-to-sample ratio is approximately 5000: 1. An aliquot (approximately 0.5-2.0 uL) of the saturated matrix solution is then allowed to dry to form the analyte-matrix mixture.

[00569] In one embodiment, the analyte-matrix mixture may be formed using the thin-layer method. A matrix homogeneous film is first formed and then the sample is then applied and may be absorbed by the matrix to form the analyte-matrix mixture.

[00570] In one embodiment, the analyte-matrix mixture may be formed using the thick-layer method. A matrix homogeneous film is formed with a nitro-cellulose matrix additive. Once the uniform nitro-cellulose matrix layer is obtained the sample is applied and absorbed into the matrix to form the analyte-matrix mixture.

[00571] In one embodiment, the analyte-matrix mixture may be formed using the sandwich method. A thin layer of matrix crystals is prepared as in the thin-layer method followed by the addition of droplets of aqueous trifluoroacetic acid, the sample and matrix. The sample is then absorbed into the matrix to form the analyte-matrix mixture.

V. Uses of Signal-Sensor Polynucleotides, Primary constructs and mmRNA of the Invention

[00572] The signal-sensor polynucleotides, primary constructs and mmRNA of the present invention are designed, in preferred embodiments, to provide for avoidance or evasion of deleterious bio-responses such as the immune response and/or degradation pathways, overcoming the threshold of expression and/or improving protein production capacity, improved expression rates or translation efficiency, improved drug or protein half life and/or protein concentrations, optimized protein localization, to improve one or more of the stability and/or clearance in tissues, receptor uptake and/or kinetics, cellular access by the compositions, engagement with translational machinery, secretion efficiency (when applicable), accessibility to circulation, and/or modulation of a cell's status, function and/or activity.

Therapeutics

Therapeutic Agents

[00573] The signal-sensor polynucleotides, primary constructs or mmRNA of the present invention, such as modified nucleic acids and modified RNAs, and the proteins translated from them described herein can be used as therapeutic or prophylactic agents. They are provided for use in medicine. For example, signal-sensor polynucleotide, primary construct or mmRNA described herein can be administered to a subject, wherein the signal-sensor polynucleotide, primary construct or mmRNA is translated *in vivo* to produce a therapeutic or prophylactic oncology-related polypeptide in the subject. Provided are compositions, methods, kits, and reagents for diagnosis, treatment or prevention of a disease or condition in humans and other mammals. The active

therapeutic agents of the invention include signal-sensor polynucleotides, primary constructs or mmRNA, cells containing polynucleotides, primary constructs or mmRNA or polypeptides translated from the signal-sensor polynucleotides, primary constructs or mmRNA.

[00574] In certain embodiments, provided herein are combination therapeutics containing one or more signal-sensor polynucleotide, primary construct or mmRNA containing translatable regions that encode for a protein or proteins that boost a mammalian subject's immunity along with a protein that induces antibody-dependent cellular toxicity.

[00575] Provided herein are methods of inducing translation of a recombinant polypeptide in a cell population using the signal-sensor polynucleotide, primary construct or mmRNA described herein. Such translation can be *in vivo, ex vivo, in culture,* or *in vitro.* The cell population is contacted with an effective amount of a composition containing the signal-sensor nucleic acid that has at least one nucleoside modification, and a translatable region encoding the recombinant oncology-related polypeptide. The population is contacted under conditions such that the signal-sensor nucleic acid is localized into one or more cells of the cell population and the recombinant oncology-related polypeptide is translated in the cell from the signal-sensor nucleic acid.

[00576] An "effective amount" of the composition is provided based, at least in part, on the target tissue, target cell type, means of administration, physical characteristics of the nucleic acid (e.g., size, and extent of modified nucleosides), and other determinants. In general, an effective amount of the composition provides efficient protein production in the cell, preferably more efficient than a composition containing a corresponding unmodified nucleic acid. Increased efficiency may be demonstrated by increased cell transfection (i.e., the percentage of cells transfected with the nucleic acid), increased protein translation from the nucleic acid, decreased nucleic acid degradation (as demonstrated, e.g., by increased duration of protein translation from a modified nucleic acid), or reduced innate immune response of the host cell.

[00577] Aspects of the invention are directed to methods of inducing *in vivo* translation of a recombinant polypeptide in a mammalian subject in need thereof. Therein, an effective amount of a composition containing a nucleic acid that has at least one

structural or chemical modification and a translatable region encoding the recombinant polypeptide is administered to the subject using the delivery methods described herein. The nucleic acid is provided in an amount and under other conditions such that the nucleic acid is localized into a cell of the subject and the recombinant polypeptide is translated in the cell from the nucleic acid. The cell in which the nucleic acid is localized, or the tissue in which the cell is present, may be targeted with one or more than one rounds of nucleic acid administration.

[00578] In certain embodiments, the administered signal-sensor polynucleotide, primary construct or mmRNA directs production of one or more recombinant polypeptides that provide a functional activity which is substantially absent in the cell, tissue or organism in which the recombinant oncology-related polypeptide is translated. For example, the missing functional activity may be enzymatic, structural, or gene regulatory in nature. In related embodiments, the administere signal-sensor polynucleotide, primary construct or mmRNA directs production of one or more recombinant oncology-related polypeptides that increases (e.g., synergistically) a functional activity which is present but substantially deficient in the cell in which the recombinant oncology-related polypeptide is translated.

[00579] In other embodiments, the administere signal-sensor polynucleotide, primary construct or mmRNA directs production of one or more recombinant polypeptides that replace an oncology-related polypeptide (or multiple oncology-related polypeptides) that is substantially absent in the cell in which the recombinant oncology-related polypeptide is translated. Such absence may be due to genetic mutation of the encoding gene or regulatory pathway thereof. In some embodiments, the recombinant oncology-related polypeptide increases the level of an endogenous oncology-related protein in the cell to a desirable level; such an increase may bring the level of the endogenous oncology-related protein from a subnormal level to a normal level or from a normal level to a super-normal level.

[00580] Alternatively, the recombinant oncology-related polypeptide functions to antagonize the activity of an endogenous protein present in, on the surface of, or secreted from the cell. Usually, the activity of the endogenous oncology-related protein is deleterious to the subject; for example, due to mutation of the endogenous oncology-

related protein resulting in altered activity or localization. Additionally, the recombinant oncology-related polypeptide antagonizes, directly or indirectly, the activity of a biological moiety present in, on the surface of, or secreted from the cell. Examples of antagonized biological moieties include lipids (e.g., cholesterol), a lipoprotein (e.g., low density lipoprotein), a nucleic acid, a carbohydrate, a protein toxin such as shiga and tetanus toxins, or a small molecule toxin such as botulinum, cholera, and diphtheria toxins. Additionally, the antagonized biological molecule may be an endogenous protein that exhibits an undesirable activity, such as a cytotoxic or cytostatic activity.

[00581] The recombinant oncology-related proteins described herein may be engineered for localization within the cell, potentially within a specific compartment such as the nucleus, or are engineered for secretion from the cell or translocation to the plasma membrane of the cell.

[00582] In some embodiments, modified signal-sensor mRNAs and their encoded oncology-related polypeptides in accordance with the present invention may be used for treatment of any of a variety of diseases, disorders, and/or conditions described herein.

Oncology-Related Applications

[00583] In one embodiment, the signal-sensor polynucleotides, primary constructs and/or mmRNA may be used in the treatment, management, characterization and/or diagnosis of cancer, a cancer-related and/or a cancer treatment-related disorder, side effect and/or condition. Such disease, disorders and conditions include, but are not limited to, adrenal cortical cancer, advanced cancer, anal cancer, aplastic anemia, bileduct cancer, bladder cancer, bone cancer, bone metastasis, brain tumors, brain cancer, breast cancer, childhood cancer, cancer of unknown primary origin, Castleman disease, cervical cancer, colon/rectal cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, fallopian tube cancer, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, gestational trophoblastic disease, Hodgkin disease, Kaposi sarcoma, renal cell carcinoma, laryngeal and hypopharyngeal cancer, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myelomonocytic leukemia, liver cancer, non-small cell lung cancer, small cell lung cancer, lung carcinoid tumor, lymphoma of the skin, malignant mesothelioma, multiple myeloma, myelodysplasia syndrome, nasal cavity and

paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma in adult soft tissue, basal and squamous cell skin cancer, melanoma, small intestine cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, Wilms tumor.

[00584] In another embodiment, the signal-sensor polynucleotides, primary constructs and/or mmRNA may be used in the treating, managing or manipulating at least one cancer-related or cancer treatment-related disorder, side effect or condition such as chemo brain, peripheral neuropathy, fatigue, depression, nausea and vomiting, pain, anemia, lymphedema, infections, second cancers caused by cancer treatment, sexual side effects, reduced fertility or infertility, ostomies, insomnia and hair loss.

[00585] In one embodiment, the signal-sensor polynucleotides, primary constructs and/or mmRNA may be used to reduce the effect of at least one symptom of cancer in a subject. The symptom may include, but is not limited to, weakness, aches and pains, fever, fatigue, weight loss, blood clots, increased blood calcium levels, low white blood cell count, short of breath, dizziness, headaches, hyperpigmentation, jaundice, erthema, pruritis, excessive hair growth, change in bowel habits, change in bladder function, long-lasting sores, white patches inside the mouth, white spots on the tongue, unusual bleeding or discharge, thickening or lump on parts of the body, indigestion, trouble swallowing, changes in warts or moles, change in new skin and nagging cough or hoarseness.

[00586] In one embodiment, the signal-sensor polynucleotides may be investigated in any number of cancer or normal cell lines. Non-limiting examples of cell lines which may be useful in these investigations include those from ATCC (Manassas, VA) including MRC-5, A549, T84, NCI-H2126 [H2126], NCI-H1688 [H1688], WI-38, WI-38 VA-13 subline 2RA, WI-26 VA4, C3A [HepG2/C3A, derivative of Hep G2 (ATCC HB-8065)], THLE-3, H69AR, NCI-H292 [H292], CFPAC-1, NTERA-2 cl.Dl [NT2/D1], DMS 79, DMS 53, DMS 153, DMS 114, MSTO-21 1H, SW 1573 [SW-1573, SW1573], SW 1271 [SW-1271, SW1271], SHP-77, SNU-398, SNU-449, SNU-182, SNU-475, SNU-387, SNU-423, NL20, NL20-TA [NL20T-A], THLE-2,

HBE135-E6E7, HCC827, HCC4006, NCI-H23 [H23], NCI-H1299, NCI-H187 [H187], NCI-H358 [H-358, H358], NCI-H378 [H378], NCI-H522 [H522], NCI-H526 [H526], NCI-H727 [H727], NCI-H810 [H810], NCI-H889 [H889], NCI-HI 155 [H1 155], NCI-H1404 [H1404], NCI-N87 [N87], NCI-H196 [H196], NCI-H21 1 [H21 1], NCI-H220 [H220], NCI-H250 [H250], NCI-H524 [H524], NCI-H647 [H647], NCI-H650 [H650], NCI-H71 1 [H71 1], NCI-H719 [H719], NCI-H740 [H740], NCI-H748 [H748], NCI-H774 [H774], NCI-H838 [H838], NCI-H841 [H841], NCI-H847 [H847], NCI-H865 [H865], NCI-H920 [H920], NCI-H1048 [H1048], NCI-H1092 [H1092], NCI-HI 105 [HI 105], NCI-H1 184 [H1 184], NCI-H1238 [H1238], NCI-H1341 [H1341], NCI-H1385 [H1385], NCI-H1417 [H1417], NCI-H1435 [H1435], NCI-H1436 [H1436], NCI-H1437 [H1437], NCI-H1522 [H1522], NCI-H1563 [H1563], NCI-H1568 [H1568], NCI-H1573 [H1573], NCI-H1581 [H1581], NCI-H1618 [H1618], NCI-H1623 [H1623], NCI-H1650 [H-1650, H1650], NCI-H1651 [H1651], NCI-H1666 [H-1666, H1666], NCI-H1672 [H1672], NCI-H1693 [H1693], NCI-H1694 [H 1694], NCI-H 1703 [H1703], NCI-H1734 [H-1734, H1734], NCI-H1755 [H1755], NCI-H1755 [H1755], NCI-H1770 [HI 770], NCI-H 1793 [H1793], NCI-H1836 [H1836], NCI-H1838 [H1838], NCI-H1869 [H1869], NCI-H1876 [H1876], NCI-H1882 [H1882], NCI-H1915 [H1915], NCI-H1930 [H1930], NCI-H1944 [H1944], NCI-H1975 [H-1975, H1975], NCI-H1993 [H1993], NCI-H2023 [H2023], NCI-H2029 [H2029], NCI-H2030 [H2030], NCI-H2066 [H2066], NCI-H2073 [H2073], NCI-H2081 [H2081], NCI-H2085 [H2085], NCI-H2087 [H2087], NCI-H2106 [H2106], NCI-H21 10 [H21 10], NCI-H2135 [H2135], NCI-H2141 [H2141], NCI-H2171 [H2171], NCI-H2172 [H2172], NCI-H2195 [H2195], NCI-H2196 [H2196], NCI-H2198 [H2198], NCI-H2227 [H2227], NCI-H2228 [H2228], NCI-H2286 [H2286], NCI-H2291 [H2291], NCI-H2330 [H2330], NCI-H2342 [H2342], NCI-H2347 [H2347], NCI-H2405 [H2405], NCI-H2444 [H2444], UMC-1 1, NCI-H64 [H64], NCI-H735 [H735], NCI-H735 [H735], NCI-H1963 [H1963], NCI-H2107 [H2107], NCI-H2108 [H2108], NCI-H2122 [H2122], Hs 573.T, Hs 573.Lu, PLC/PRF/5, BEAS-2B, Hep G2, Tera-1, Tera-2, NCI-H69 [H69], NCI-H128 [H128], ChaGo-K-1, NCI-H446 [H446], NCI-H209 [H209], NCI-H146 [H146], NCI-H441 [H441], NCI-H82 [H82], NCI-H460 [H460], NCI-H596 [H596], NCI-H676B [H676B], NCI-H345 [H345], NCI-H820 [H820], NCI-H520 [H520], NCI-H661 [H661], NCI-H510A [H510A, NCI-H510], SK-HEP-1, A-427,

Calu-1, Calu-3, Calu-6, SK-LU-1, SK-MES-1, SW 900 [SW-900, SW900], Malme-3M, and Capan-1.

[00587] In one embodiment, the signal-sensor polynucleotides described herein may be investigated in human lung adenocarcinoma. As a non-limiting example, a signal-sensor polynucleotide encoding constitutively active caspase 3 fully modified with 5-methylcytidine and 1-methylpseudouridine or fully modified with 1-methylpseudouridine may be delivered to cultured human lung adenocarcinoma A549 cells (see e.g., the experiment outlined in Example 53). As another non-limiting example, a signal-sensor polynucleotide encoding constitutively active caspase 6 fully modified with 5-methylcytidine and 1-methylpseudouridine or fully modified with 1-methylpseudouridine may be delivered to cultured human lung adenocarcinoma A549 cells (see e.g., the experiment outlined in Example 53).

[00588] In another embodiment, the signal-sensor polynucleotides described herein may be investigated in human hepatocellular carcinoma. As a non-limiting example, a signal-sensor polynucleotide encoding constitutively active caspase 3 fully modified with 5-methylcytidine and 1-methylpseudouridine or fully modified with 1-methylpseudouridine may be delivered to human hepatocellular carcinoma Hep3B cells (see e.g., the experiment outlined in Example 54).

[00589] In one embodiment, the signal-sensor polynucleotides may be investigated in an animal model. As a non-limiting example, the animal model may be for lung cancer such as the lung cancer model of Fukazawa et al (Anticancer Research, 2010; 30: 4193-4200) where a congenic mouse is created by crossing a ubiquitously expressing dominant negative Myc (Omomyc) mouse with a KRAS mutation- positive lung cancer model mouse. In the presence of Omomyc, lung tumors caused by the expression of mutated KRAS regresses in the congenic mouse, indicating that Omomyc caused tumor cell death of KRAS mutation-positive lung cancer.

[00590] As another non-limiting example, Human lung cancer xenografts are also prepared by the method of Fukazawa where human lung cancer xenografts are established in 4-week-old female BALB/C nude mice (Charles River Laboratories Japan, Kanagawa, Japan) by subcutaneous inoculation of 4x106 A549 cells into the dorsal flank. The mice are randomly assigned into six groups (n=6/group). After the tumors reach a

diameter of about 0.5 cm (approximately 6 days after tumor inoculations), each group of mice are injected with 100 µï solution containing PBS, 5x1010 vp of control or signal-sensor polynucleotide into the dorsalflank tumor for the selected dosing regimen. Animals are then observed closely and survival studiesor other analyses are performed.

[00591] In one embodiment, the signal-sensor polynucleotides may be investigated in a transgenic animal model. As a non-limiting example, the transgenic animal model is a LSL-KRAS^{G12D}: TRE Omomyc:CMV rtTA triple transgenic model which involves the use of an adenovirus expressing Cre recombinase which is administered via inhalation to induce oncogene expression via excision of the floxed STOP codon, and ubiquitous Omomyc expression is controlled via doxycycline. The model is reported in Soucek et al. (Nature, 1-5 (2008)). As another non-limiting example, the mice of Soucek may be crossed with the LSLKRAS^{G12D} single transgenic mice (Jackson Laboratories) and may be used for inhalation delivered or otherwise lung-delivered studies of signal-sensor polynucleotides expressing MYC inhibitor **D** or other oncology related polypeptide described herein.

[00592] In another embodiment, the signal-sensor polynucleotides may be investigated in a mouse-in-mouse model such as, but not limited to a model which is akin to the p53-/-:c-Myc overexpressing HCC model of Zender (Cell. 2006 June 30; 125(7): 1253-1267).

[00593] In one embodiment, the signal-sensor polynucleotides may be investigated in a Nongermline genetically engineered mouse model (NGEMM). As a non-limiting example, the design of mouse-in-mouse model may involve starting with the WT or tumor suppressor deleted (such as p53 -/-) 129 Sv/Ev Mm ES cell clone; introduction of liver activated protein (LAP) promoter directed tetracycline transactivator (tTA) and tetO-luciferase for liver specific imaging; freezing the resulting LAP-tTA: tetO-luciferase clones to be used for c-Myc as well as other liver relevant programs oncogene; adding tetO driven oncogene, e.g. tetOcMyc; Freeze resulting LAP-tTA: tetO-luciferase: tetO-MYC clones; injecting resulting ES clones into C57B1/6 blastocytes and implant in pseudo pregnant mothers whereby the resulting chimeric animals are the tumor model upon removal of doxycycline (i.e. Tet- Off). The type of model will ideally evince inducible nodules of c-Myc-driven, luciferase-expressing HCC surrounded by normal hepatocytes.

[00594] In another embodiment, the signal-sensor polynucleotides may be investigated in Orthotopic HCC models using the HEP3B cell lines in mice (Crown Bio).

[00595] As a non-limiting example, any of the animal models described above may be used to investigate a signal-sensor polynucleotide encoding MYC inhibitor D. The study may also include a signal-sensor polynucleotide encoding a negative control such as, but not limited to, an untranslatable mRNA for MYC inhibitor D and a vehicle only delivery. The animal may be evaluated for gene expression, tumor status and/or for any of the hallmarks that are generally associated with cancer phenotypes or genotypes.

[00596] As another non-limiting example, any of the animal models described above may be used to investigate a signal-sensor polynucleotide encoding dominant negative hTERT. The study may also include a signal-sensor polynucleotide encoding a negative control such as, but not limited to, an untranslatable mRNA for dominant negative hTERT and a vehicle only delivery. The animal may be evaluated for gene expression, tumor status and/or for any of the hallmarks that are generally associated with cancer phenotypes or genotypes.

[00597] As another non-limiting example, any of the animal models described above may be used to investigate a signal-sensor polynucleotide encoding dominant negative survivin. The study may also include a signal-sensor polynucleotide encoding a negative control such as, but not limited to, an untranslatable mRNA for dominant negative survivin and a vehicle only delivery. The animal may be evaluated for gene expression, tumor status and/or for any of the hallmarks that are generally associated with cancer phenotypes or genotypes.

[00598] In one embodiment, signal-sensor polynucleotides may include at least one miRNA-binding site in the 3'UTR in order to direct cytotoxic or cytoprotective mRNA therapeutics to specific cells such as, but not limited to, normal and/or cancerous cells in an animal model described herein. As a non-limiting example, a strong apoptotic signal and at least one miR-122a binding site is encoded by the signal-sensor polynucleotide where the at least one miR-122a binding site is located in the 3'UTR. As another non-limiting example, apoptosis inducing factor short isoform (AIFsh) and at least one miR-122a binding site is encoded by the signal-sensor polynucleotide where the at least one miR-122a binding site is located in the 3'UTR. As yet another non-limiting example,

constitutively active (C.A.) caspase 6 and at least one miR-122a binding site is encoded by the signal-sensor polynucleotide where the at least one miR-122a binding site is located in the 3'UTR. As another non-limiting example, HSVl-tk and at least one miR-122a binding site is encoded by the signal-sensor polynucleotide where the at least one miR-122a binding site is located in the 3'UTR.

[00599] In another embodiment, signal-sensor polynucleotides may include three miRNA-binding sites in the 3'UTR in order to direct cytotoxic or cytoprotective mRNA therapeutics to specific cells such as, but not limited to, normal and/or cancerous cells in an animal model described herein. As a non-limiting example, a strong apoptotic signal and three miR-122a binding sites are encoded by the signal-sensor polynucleotide where the three miR-122a binding sites are located in the 3'UTR. As another non-limiting example, apoptosis inducing factor short isoform (AIFsh) and three miR-122a binding sites are encoded by the signal-sensor polynucleotide where the three miR-122a binding sites are located in the 3'UTR. As yet another non-limiting example, constitutively active (C.A.) caspase 6 and three miR-122a binding sites are encoded by the signal-sensor polynucleotide where the three miR-122a binding sites are located in the 3'UTR. As another non-limiting example, HSVl-tk and and three miR-122a binding sites are encoded by the signal-sensor polynucleotide where the three miR-122a binding sites are located in the 3'UTR.

Common Categories of Cancer

Brain Cancer

[00600] Brain cancer is the growth of abnormal cells in the tissues of the brain usually related to the growth of malignant brain tumors. Brain tumors grow and press on the nearby areas of the brain which can stop that part of the brain from working the way it should. Brain cancer rarely spreads into other tissues outside of the brain. The grade of tumor, based on how abnormal the cancer cells look under a microscope, may be used to tell the difference between slow- and fast-growing tumors. Grade I tumors grow slowly, rarely spreads into nearby tissues, has cells that look like normal cells and the entire tumor may be removable by surgery. Grade II tumors also grow slowly but may spread into nearby tissue and may recur. Grade III tumors grow quickly, is likely to spread into nearby tissue and the tumor cells look very different from normal cells. Grade IV, high-

grade, grows and spreads very quickly and there may be areas of dead cells in the tumor. Symptoms of brain cancer may include, but are not limited to, morning headache or headache that goes away after vomiting, frequent nausea and vomiting, vision, hearing, and speech problems, loss of balance and trouble walking, weakness on one side of the body, unusual sleepiness or change in activity level, unusual changes in personality or behavior, seizures.

[00601] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with brain cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may be diagnosed with brain cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with brain cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest.

Breast Cancer

[00602] Breast cancer forms in the tissues of the breast, of both men and women, such as, but not limited to, the ducts and the lobules. The most common type of breast cancer is ductal carcinoma which begins in the cells of the ducts. Lobular cancer, which begins in the lobes or lobules, is often found in both breasts. An uncommon type of breast cancer, inflammatory breast cancer, causes the breast to be warm, red and swollen. Hereditary breast cancer makes up approximately 5-10% of all breast cancer and altered genes are common in some ethnic groups making that enthic group more susceptible to breast cancer. Symptoms of breast cancer include, but are not limited to, a lumpm or thickening in or near the breast or in the underarm area, change in the size or shape of the breast, dimple or puckering in the skin of the breast, inward turned nipple of the breast, fluid from the nipple which is not breast milk, scaly, red or swollen skin on the breast,

nipple, or areola, and dimples in the breast that look like the skin of orange (peau dOrange).

[00603] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with breast cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may be diagnosed with breast cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with breast cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest.

Cervical Cancer

[00604] Cervical cancer forms in the tissues of the cervic and is usually slow-growing. The cause of cervical cancer usually related to the human papillomavirus (HPV) infection. Although cervical cancer may not not show any signs, possible symptoms may include, but are not limited to, vaignal bleeding, unusal vaginal discharge, pelvic pain and pain during sexual intercourse.

[00605] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with cervical cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may be diagnosed with cervical cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to

reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with cervical cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest.

Esophageal Cancer

[00606] Esophageal cancer is cancer that forms in the tissues lining the esophagus. There are two common types of esophageal cancer which are named for the type of cells that become malignant. Squamous cell carcinoma is cancer that forms in the thin, flat cells lining the esophagus (also called epidermoid carcinoma). Cancer that begins in the glandular (secretory) cells which produce and release fluids such as mucus is called adneocarcinoma. Common symptoms associated with esophageal cancer include, but are not limited to, painful or difficult swallowing, weight loss, pain behind the breastbone, hoarseness and cough, and indigestion and heartburn.

[00607] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with esophageal cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may be diagnosed with esophageal cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with esophageal cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide.

Familial Cancer Syndrome

[00608] Familial cancer syndrome describes the genetic predisposition of a subject to develop cancer. 5-10% of all cancers are hereditary and are passed on through specific in specific genes passed from one blood relative to another. Subjects that inherit one of these gene changes may have a higher likelihood of developing cancer within their

lifetime. Familial cancer syndrome includes disorder such as, but not limited to, Ataxia Telangiectasia, Basal Cell Nevus Syndrome, Nevoid Basal Cell Carcinoma Syndrome, Gorlin Syndrome, Beck-with Wiedemann Syndrome, Birt-Hogg-Dube Syndrome, Bloom Syndrome, hereditary breast and/or ovarian cancer, Carney Complex, Types I and II, Familial Chordoma, Colon Cancer, Hereditary Nonpolyposis-Lynch Syndrome, Costello Syndrome, Facio-Cutaneous-Skeletal Syndrome, Cowden Syndrome, Dyskeratosis Congenita, Tylosis with Esophaeal Cancer, Keratosis Palmaris et Plantaris with Esophageal Cancer, Howel-Evans Syndrome, Herediatary Multiple Exostosis, Fanconi Anemia, Hereditary Diffuse Gastric Cancer, Gastrointestinal Stromal Tumor, Multiple Gastrointestinal Stromal Tumor, Familial Hyperparathyroidism, Acute Myeloid Leukemia, Familial Leukemia, Chronic Lymphocytic Leukemia, Li-Fraumeni Syndrome, Hodgkin Lymphoma, Non-Hodgkin Lymphoma, Hereditary Multiple Melanoma, Mosaic Varigated Aneuploidy, Multple Endocrine Neoplasia Type I, Type 2A and 2B, Familial Medullary Thyroid Cancer, Familial Mulitple Myeloma, Hereditary Neuroblastoma, Neurofibromatosis Type 1 and 2, Nijmegen Breakage Syndrome, Hereditary Pancreatic Cancer, Hereditary Paraganglioma, Peutz-Jeghers Syndrome, Familial Adenomatous Polyposis, Familial Juvenile Polyposis, MYH-Associated Polyposis, Hereditary Prostate Cancer, Hereditary Renal Cell Carcinoma with Multiple Cutaneous and Uterine Leiomyomas, Hereditary Renal Cell Carcinoma, Hereditary Papillary Renal Cell Carcinoma, Rhabdoid Predisposition Syndrome, Rothmund-Thomson Syndrome, Simpson-Golabi-Behmel Syndrome, Familial Testicular Germ Cell Tumor, Familial Non-medullary Thyroid Carcinoma, Tuberous Sclerosis Complex, von Hippel-Lindau Syndrome, Familial Waldenstrom Macroglobulinemia, Werner Syndrome, Familial Wilms Tumor and Xeroderma Pigmentosum.

[00609] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with Familial cancer syndrome by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may

be diagnosed with Familial cancer syndrome by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with Familial cancer syndrome by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest.

Leukemia

[00610] Leukemia is a form of cancer that starts in blood-forming tissue such as the bone marrow which can cause a large number of blood cells to be produced and enter the blood stream. Leukemia can also spread to the central nervous system and cause brain and spinal cord cancer. Types of leukemia include, but are not limited to, adult acute lymphoblastic, childhood acute lymphoblastic, aduct acute myeloid, chronic lymphocytic, chronic myelogenous and hairy cell. Non-limiting examples of symptoms of leukemia include weakness or feeling tired, fever, easy bruising or bleeding, petechiae, shortness of breath, weight loss or loss of appetite, pain in the bones or stomach, pain or feeling of fullness below the ribs, and painless lumps in the neck, underarm, stomach or groin.

[00611] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with leukemia by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may be diagnosed with leukemia by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with leukemia by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest.

Liver Cancer

[00612] There are two types of liver cancer, primary liver cancer which forms in the tissue of the liver and secondary liver cancer, or metastatic liver cancer, that spreads to the liver from another part of the body. Possible symptoms of liver canver include, but are not limited to, a hard lump on the right side just below the rib cage, discomfort in the upper abdomen on the right side, pain around the right shoulder blade, unexplained weight loss, jaundice, unusual tiredness, nausea and loss of appetide.

[00613] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with liver cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may be diagnosed with liver cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with liver cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest.

Hepatocellular carcinoma

[00614] The c-myc protein is a multifunctional bHLHZip transcription factor with critical roles in normal cellular processes and aberrantly regulated in the majority of human cancers. c-, N- and L-Myc are family members that can dimerize with partners such as Max, Mad and Miz-1 . The protein is implicated in the transactivation and repression of a vast number of proposed transcriptional targets and recent work has demonstrated a role for Myc as a"transcriptional amplifier" of otherwise transactivated genes in developing cancers. It has a well established function in cancer cell proliferation, growth, biosynthetic metabolism, ribogenesis and translation and possibly a non-redundant node through whichoncogenic signals must navigate.

[00615] MYC inhibitor **D** (also known as Omomyc) is a unique dominant-negative 90 a.a. protein comprised of the human c-Myc oligomerization domain with 4 introduced

mutations E57T, E64I, R70Q, R71N (Soucek *et al, Oncogene,* 1998;17, 2463-2472). Importantly, it exhibits selectivity in binding and inhibitory capability: binding c-Myc, N-Myc, Max and Miz-1. It also prevents E-box mediated transactivation while retaining Miz-1 directed transrepression. The therapeutic potential of MYC inhibitor D has been specifically exhibited *in vivo* where transgenic expression of OMOMYC blocked MycERTAM induced keratinocyte proliferation (Soucek *et al, CDD* 2004; 11, 1038-1045); transgenic Omomyc prevented the establishment and induced the regression of forming and mature lung tumors, respectively, in the LSL-KrasG12D mouse model with reversible toxicity (Soucek *et al, Nature* 2008, 455, 679-683); transgenic Omomyc prevents tumor formation and regresses established tumors in the RIP1-TAG2 model of pancreatic neuroendocrine cancer with controllable side effects, and further shows a role for cancer cell Myc in the maintenance of a permissive tumor microenvironment (Sodir *et al, Genes and Development* 201 1, 25, 907-916); and it was reported "that Omomyc induces cell death of KRAS-mutated human lung adenocarcinoma A549 cells in vitro and in vivo" (Fukazawa *et al, Anticancer Res,* 2010, 30, 4193-4200).

[00616] Although it stands to reason that the inhibition of oncogenic c-Myc via the directed expression of MYC inhibitor D might prove to be an effective therapy in at least a subset of HCCs, proof of concept in HCC remains to be demonstrated.

[00617] In some embodiments, the present invention includes signal-sensor polynucleotides encoding MYC inhibitor D as the oncology-related polypeptide; with or without a sensor sequence for the treatment of hepatocellular carcinoma (HCC). The studies of HCC may be performed in any of the subclasses of HCC cell lines as described by Hoshida et al (Cancer Research 2009; 69: 7385-7392). These include S2 cells wich have higher TGF-beta and WNT signaling and demonstrate and associated with a greater risk of early recurrence, S2 which exhibit increased myc and AKT expression and the highest level of alpha feto-protein or S3 which retain the hepatocyte like phenotype. SI and S2 types have also been shown to exhibit increased E2F1 and decreased p53 expression; while S2 alone has shown decreased levels of interferon. S1 cell lines include SNU-387, SNU-423, SNU- 449, SNU-475, SNU-182, SK-Hepl, HLE, HLF, and Focus, whereas S2 cell lines include Huh-1, Huh-6, Huh-7, HepG2, Hep3B, Hep3B-TR, Hep40, and PLC/PRF/5 cells.

Lung Cancer

[00618] Lung cancer forms in the tissues of the lung usually in the cells lining the air passages and is classified as either small cell lung cancer or non-small cell lung cancer. There are two types of small cell lung cancer, small cell carcinoma and combined small cell carcinoma. The types of on-small cell lung cancer are squamous cell carcinoma (cancer begins in the squamous cells), large cell carcinoma (cancer may begin in several types of cells) and adenocarcinoma (cancer begins in the cells that line the alveoli and in cells that make mucus). Symptoms of lung cancer include, but are not limited to, chest discomfort or pain, cough that does not go away or gets worse over time, trouble breathing, wheezing, blood in the sputum, hoarseness, loss of appetite, weight loss for no known reason, feeling very tired, trouble swallowing and swelling in the face and/or veins in the neck.

[00619] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with lung cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may be diagnosed with lung cancer by administereing to said subject an isolated polynucleotide encoding a polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with lung cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest.

Lymphoma

[00620] Lymphoma is cancer that beings in the cells of the immune system. Subjects who have Hodgkin lymphoma have a cell called Reed-Sternberg cell and non-Hodgkin lymphoma includes a large group of cancers of immune system cells. Examples of Lymphoma include, but are not limited to, painless, swollen lymph nodes in the neck,

underarm or groin, fever for no known reason, drenching night sweats, weight loss for no known reason, itchy skin and fatigue.

[00621] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with lymphoma by administereing to said subject an isolated polynucleotide encoding a polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may be diagnosed with lymphoma by administereing to said subject an isolated polynucleotide encoding a polypeptide of interest. In one embodiment, the polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with lymphoma by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest.

Ovarian Cancer

[00622] Ovarian cancer is cancer which forms in the tissues of the ovary which are either ovarian epithelial carcinomas (begins on the surface of the ovary) or malignant germ cell tumors (cancer that begins in the egg cells). Symptoms of ovarian cancer include, but are not limited to, pain or swelling in the abdomen, pain in the pelvis, gastrointestinal problems such as gas, bloating, or constipation and vaginal bleeding after menopause.

[00623] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with ovarian cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or signal-sensor mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may be diagnosed with ovarian cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one

embodiment, the signal-sensor polynucleotides, primary constructs or signal-sensor mmRNA of the present invention may be used to reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with ovarian cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest.

Prostate Cancer

[00624] Prostate that forms in the tissue of the prostate mainly affects older men. Non-limiting examples of prostate cancer include, but are not limited to, weak or interrupted flow of urine, frequent urination, trouble urinating, pain or burning during urination, blood in the urine or semen, pain in the back, hips or pelvis that does not go away and painful ejaculation.

[00625] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with prostate cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may be diagnosed with prostate cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with prostate cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest.

Testicular Cancer

[00626] Testicular cancer forms in the tissues of one or both testicles and is most common in young or middle-aged men. Most testicular cancers being in germ cells and are called testicular germ cell tumors. There are two types of testicular germ cell tumors called seminomas and nonseminomas. Common symptoms of testicular cancer include, but are not limited to, a painless lump or swelling in either testicle, change in how the

testicle feels, dull ache in the lower abdomen or the groin, sudden build-up of fluid in the scrotum and pain or discomfort in a testicle or in the scrotum.

[00627] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with testicular cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may be diagnosed with testicular cancer by administereing to said subject an isolated signal-sensor polynucleotide encoding an oncology-related polypeptide of interest. In one embodiment, the polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with testicular cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest.

Throat Cancer

[00628] Throat cancer forms in the tissues of the pharynx and includes cancer of the nasopharynx (nasopharyngeal cancer), oropharynx (oropharyngeal cancer), hypopharynx (hypopharyngeal cancer), and larynx (laryngeal cancer). Common symptoms of throat cancer include, but are not limited to, a sore throat that does not go away, ear pain, lump in the neck, painful or difficulty swallowing, change or hoarseness in the voice, trouble breathing or speaking, nosebleeds, trouble hearing, pain or ringing in the ear, headaches, dull pain behind the breast bone, cough and weight loss for no reason.

[00629] In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to treat a disease, disorder and/or condition in a subject who has been diagnosed or may be diagnosed with throat cancer by administereing to said subject an isolated polynucleotide encoding an oncology-relateda polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce, eliminate, or prevent tumor growth in a subject who has been diagnosed or may be diagnosed with throat cancer by administereing to said subject an isolated polynucleotide encoding an

oncology-related polypeptide of interest. In one embodiment, the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention may be used to reduce and/or ameliorate at least one symptom of cancer in a subject who has been diagnosed or may be diagnosed with throat cancer by administereing to said subject an isolated polynucleotide encoding an oncology-related polypeptide of interest.

Inhibition of Hypoxia-inducible factors (HIFs)

[00630] Hypoxia-inducible factors (HIFs) control cellular adaptation to oxygen deprivation. Cancer cells engage HIFs to sustain their growth in adverse conditions, thus promoting a cellular reprograming that includes metabolism, proliferation, survival and mobility. HIFs overexpression in human cancer biopsies correlates with high metastasis and mortality.

[00631] HIFs regulate genes related to metabolism such as GLUT1, GLUT3, ALDOA, ENOl, GAPDH, HKl, HK2, PFKL, PGKl, PKM2, LDHA , proliferation such as IGF-2, TGFA, VEGFA, survival such as TERT, NANOG, OCT4 and cell migration-invasion such as ZEB1, ZEB2, SNAI2, MMP14, MMP9, AMF, MET, PTHrP. (Keith , et al Nat Rev Cancer 2012; 12:9-22).

[00632] In one embodiment, one or more signal-sensor polynucleotides may be administered to the cancer cell to investigate the destabilization of cancer, The selection of the sequence, dose or administrative route is optionally informed by diagnostic evaluation of the cell, tumor, tissue or organism including, but not limited to, expression profiling of the cancer, metabolic evaluation (hypoxic, acidotic), apoptotic vs. survival profiling, cell cycle vs. senescent profiling, immune sensitivities, and/or evaluation of stromal factors.

[00633] In one embodiment, the signal-sensor polynucleotides may encode either or both of the oncology related polypeptides, CITED4 and SHARP 1. The signal-sensor polynucleotides are then administered where the administration of either or both results in the inhibition of the transcriptome of HIF-1 alpha in cancer cells. Suppression of HIF1-alpha gene regulated expression occurs upon administration with higher suppression when both polynucleotides are administered together.Reporter constructs such as luciferase under HIF1 -alpha are used in the manner similar to the methods disclosed in van de Sluis et al, (J Clin Invest. 2010;120(6):2119-2130). It is known that both CITED4

and SHARP 1 expression results in decreased HIF1 -alpha and concomitant reduction in HIF1 -alpha regulated gene expression. Cell death and/or proliferation may also be evaluated in order to determine the effectiveness of the signal-sensor polynucleotide.

[00634] In another embodiment, additional experiments can be conducted using a cancer cell line where CITED4 and SHARP 1 are themselves down regulated either under hypoxic conditions. A positive result woudl demonstrate that specifically targeting the metabolic profile (in this case hypoxic-adaptations of CITED4 and SHAPR1) with replacement of native proteins via signal-sensor polynucleotides can directly impact the transcriptome and survival advantage of cancer cells with this profile. Further, the data could show that the relative impact of signal-sensor polynucleotide vs. vehicle under hypoxic conditions was more significant for cancer cells than for normal cells. (i.e., the cancer cells have a disproportionate survival advantage based on their CITED4+SHARP1 down regulation) that makes them more sensitive to the replacement of this protein then a normal cell is to overproduction of it. It is understood that a cancer cell will likely be experiencing hypoxic conditions and that a normal cell under normoxic conditions might be able to tolerate CITED4 and SHARP 1 over expression because the normal cell is not dependent on HIF1 alpha transctiptome for survival advantage.

[00635] In one embodiment, *in vivo* experiments are performed according to the design of the *in vitro* experiments where the animal model is one evincing metastasis in the cancer setting because HIF-1 alpha appears to confer the largest portion of its advantage in metastasis. Animals are administered the signal-sensor polynucleotide compared to no treatment or a control polynucleotide. Animal cells, tissues and/or organs are then evaluated for alterations in gene expression profiles or transcriptome levels.

Titration Between Cofactors

[00636] Experiments may be conducted in order to titrate the binding affinity between two cofactors. As used herein, the term "titrate" refers to a method whereby one or more factors are introduced systematically (such as at increasing levels or wherein the one or more factors are systematically modified) to a solution, scenario or series thereof in order to assess a property of interest. In this embodiment, the property of interest is the binding affinity between two cofactors. In one embodiment, constructs encoding the two cofactors are obtained and/or synthesized and a series of mutant constructs are prepared

and/or synthesized. Mutant constructs encode cofactor mutants that may include truncated mutants (mutant proteins lacking one or more amino acids from either the N- or C-terminal domains), mutants with regional deletions [proteins wherein internal regions (comprising one or more amino acids) of the protein are absent], mutants with single amino acid substitutions (wherein a normally expressed amino acid is replaced with an alternative amino acid), mutants with one or more additional amino acids added internally or at either terminus, mutants with regional substitutions [proteins wherein internal regions (comprising one or more amino acids) of the protein are substituted with alternative regions (comprising one or more amino acids) and/or combinations of any of these. Mutant constructs are mutated randomly or subjected to targeted mutation based on existing knowledge of the molecular interactions necessary for binding between the two cofactors being investigated.

[00637] In some embodiments, a series of mutant proteins are designed such that the mutations follow a progressive pattern along the polypeptide chain. Such series may allow for a better understanding of a particular aspect or feature of the interaction between cofactors. A mutant series may include, for example, the production of a series of mutants, each with a single amino acid substitution, wherein each mutant has a different amino acid along it's polypeptide sequence mutated (e.g. alanine is substituted, thereby eliminating the influence of an amino acid side chain at each position). In another example, a series of mutants are designed such that the mutants in the series comprise truncations of increasing size. In another example, amino acids capable of being post-translationally modified (e.g. phosphorylated, acetylated, ubiquitinated, glycosylated, etc.) in a similar manner may be mutated along the polypeptide sequence in a series of mutants.

[00638] For titration experiments with mutant cofactors, a baseline affinity between the two cofactors is established by combining both cofactors under conditions favorable for binding and the binding affinity between the cofactors is assayed. Binding affinity may be assessed using any of a variety of methods known in the art. Such methods may include, but are not limited to Western blot analysis, immunoprecipitation, enzyme-linked immunosorbent assay (ELISA), fluorescence resonance energy transfer (FRET), fluorescence recovery after photobleaching (FRAP), fluorescence polarization

technologies and/or surface plasmon resonance (SPR) based technologies. For titration, according to one method, a mutant series of one or both cofactors are combined with the two unmutated cofactors (to allow for binding competition between the wild type and mutated proteins). Changes in affinity between the two cofactors in the presence of increasing concentrations of different mutants are assessed and compared and/or plotted against the specific mutations present in the series of mutants that are competing for binding. Alternatively, mutant cofactors in a series are individually combined with a corresponding unmutated binding partner and assessed for binding affinity. Increasing concentrations of the wild type cofactor (corresponding to the mutant cofactor) are introduced and changes in binding between the mutant cofactors and the corresponding unmutated binding partner are assessed. Comparisons are made between the resulting binding curves and the binding curves of other mutants tested.

[00639] In some embodiments, titration of the binding affinity between two cofactors is assessed in the presence or absence of increasing concentrations of a third factor. Such a third factor may be an inhibitor or activator of binding between the two cofactors. A series of mutants, as described above, may be generated for a third factor and such a series may be used in titration experiments to assess the effect of mutations on binding between the two cofactors.

[00640] Information obtained from titration experiments may be used to design modified mRNA molecules to encode factors that modulate the interaction between cofactors.

[00641] In some embodiments, titration experiments are carried out wherein the binding affinity between HlFl subunits (HlFl - alpha, HIF2-alpha and ARNT) and/or mutated HlFl subunits and/or other proteins that interact with HlFl is assessed. Titration experiments may utilize mutant series generated using constructs for one or more of HlFl -alpha, HIF2-alpha, ARNT and/or a third interacting factor. In some embodiments, a mutant series is generated for HlFl -alpha. HlFl -alpha and HIF2-alpha are hyrdroxylated by HIF hydroxylase enzymes under normal levels of oxygen in the cell, facilitating degredation and/or blocking transcriptional activity. Hyrdorxylation decreases as oxygen levels drop, allowing HIF 1-alpha and/or HIF2-alpha to associate with their cofactor, ARNT leading to elevated expression of genes comprising HIF-response elements

(HREs) (Keith, B. et al., HIFl a and HIF2a: sibling rivalry in hypoxic tumour growth and progression. Nat Rev Cancer. 201 1 Dec 15; 12(l):9-22). In one embodiment, HIFl-alpha mutant series are generated wherein mutations in the series progressively eliminate one or more hydroxylation sites along the polypeptide chain (including, but not limited to proline 402, proline 564 and/or asparagine 803), thereby modulating stability and/or transcriptional activity in mutant versions of HIFl-alpha. In another embodiment, an alternative cofactor, HIF2-alpha is used to generate a mutant series. Such a mutant series may progressively eliminate one or more hydroxylation sites along the polypeptide chain (including, but not limited to proline 405, proline 531 and/or asparagine 847), thereby modulating stability and/or transcriptional activity in mutant versions of HIF2-alpha. In another embodiment, HIFl-alpha and/or HIF2-alpha mutant series are generated that progressively mutate regions necessary for interaction with ARNT, thereby creating mutants with altered abilities to bind ARNT and modulate HIF-dependent gene expression. In another embodiment, ARNT mutant series are generated that progressively mutate regions necessary for interactions with other HIF subunits, thereby creating mutants with altered abilities to bind HIF subunits and modulate HIF-dependent gene expression.

[00642] In some embodiments, mutant series are generated for Von Hippel-Landau tumor suppressor protein (pVHL). This protein binds hydroxylated HIFl-alpha and HIF2-alpha, facilitating their ubiquitination and degradation. In one embodiment, mutant series are generated that progressively mutate regions necessary for interaction with HIFl subunits, thereby creating mutants with altered abilities to bind HIFl subunits and modulate HIF-dependent gene expression.

[00643] Non-limiting examples of transcript and polypeptide sequences which may be used for the titration experiments are shown in Table 27 (transcript) and Table 28 (polypeptide).

VI. Kits and Devices

Kits

[00644] The invention provides a variety of kits for conveniently and/or effectively carrying out methods of the present invention. Typically kits will comprise sufficient

amounts and/or numbers of components to allow a user to perform multiple treatments of a subject(s) and/or to perform multiple experiments.

[00645] In one aspect, the present invention provides kits comprising the molecules (signal-sensor polynucleotides, primary constructs or mmRNA) of the invention. In one embodiment, the kit comprises one or more functional antibodies or function fragments thereof.

[00646] Said kits can be for oncology-related protein production, comprising a first signal-sensor polynucleotide, primary construct or mmRNA comprising a translatable region. The kit may further comprise packaging and instructions and/or a delivery agent to form a formulation composition. The delivery agent may comprise a saline, a buffered solution, a lipidoid or any delivery agent disclosed herein.

[00647] In one embodiment, the buffer solution may include sodium chloride, calcium chloride, phosphate and/or EDTA. In another embodiment, the buffer solution may include, but is not limited to, saline, saline with 2mM calcium, 5% sucrose, 5% sucrose with 2mM calcium, 5% Mannitol, 5% Mannitol with 2mM calcium, Ringer's lactate, sodium chloride, sodium chloride with 2mM calcium. In a futher embodiment, the buffer solutions may be precipitated or it may be lyophilized. The amount of each component may be varied to enable consistent, reproducible higher concentration saline or simple buffer formulations. The components may also be varied in order to increase the stability of modified RNA in the buffer solution over a period of time and/or under a variety of conditions. In one aspect, the present invention provides kits for oncology-related protein production, comprising: signal-sensor polynucleotide, primary construct or mmRNA comprising a translatable region, provided in an amount effective to produce a desired amount of an oncology-related protein encoded by the translatable region when introduced into a target cell; a second signal-sensor polynucleotide comprising an inhibitory nucleic acid, provided in an amount effective to substantially inhibit the innate immune response of the cell; and packaging and instructions.

[00648] In one aspect, the present invention provides kits for oncology-related protein production, comprising signal-sensor polynucleotide, primary construct or mmRNA comprising a translatable region, wherein the signal-sensor polynucleotide exhibits reduced degradation by a cellular nuclease, and packaging and instructions.

[00649] In one aspect, the present invention provides kits for oncology-related protein production, comprising signal-sensor polynucleotide, primary construct or mmRNA comprising a translatable region, wherein the polynucleotide exhibits reduced degradation by a cellular nuclease, and a mammalian cell suitable for translation of the translatable region of the first nucleic acid.

Devices

[00650] The present invention provides for devices which may incorporate signal-sensor polynucleotides, primary constructs or mmRNA that encode polypeptides of interest. These devices contain in a stable formulation the reagents to synthesize a signal-sensor polynucleotide in a formulation available to be immediately delivered to a subject in need thereof, such as a human patient.

[00651] In some embodiments the device is self-contained, and is optionally capable of wireless remote access to obtain instructions for synthesis and/or analysis of the generated signal-sensor polynucleotide, primary construct or mmRNA. The device is capable of mobile synthesis of at least one signal-sensor polynucleotide, primary construct or mmRNA and preferably an unlimited number of different signal-sensor polynucleotides, primary constructs or mmRNA. In certain embodiments, the device is capable of being transported by one or a small number of individuals. In other embodiments, the device is scaled to fit on a benchtop or desk. In other embodiments, the device is scaled to fit into a suitcase, backpack or similarly sized object. In another embodiment, the device may be a point of care or handheld device. In further embodiments, the device is scaled to fit into a vehicle, such as a car, truck or ambulance, or a military vehicle such as a tank or personnel carrier. The information necessary to generate a modified signal-sensor mRNA encoding oncology-related polypeptide of interest is present within a computer readable medium present in the device.

[00652] In one embodiment, a device may be used to assess levels of an oncology-related protein which has been administered in the form of signal-sensor polynucleotide, primary construct or mmRNA. The device may comprise a blood, urine or other biofluidic test.

[00653] In some embodiments, the device is capable of communication (e.g., wireless communication) with a database of nucleic acid and polypeptide sequences which may be

signal-sensor nucleic acid and oncology-related polypeptide seqeunces. The device contains at least one sample block for insertion of one or more sample vessels. Such sample vessels are capable of accepting in liquid or other form any number of materials such as template DNA, nucleotides, enzymes, buffers, and other reagents. The sample vessels are also capable of being heated and cooled by contact with the sample block. The sample block is generally in communication with a device base with one or more electronic control units for the at least one sample block. The sample block preferably contains a heating module, such heating molecule capable of heating and/or cooling the sample vessels and contents thereof to temperatures between about -20C and above +100C. The device base is in communication with a voltage supply such as a battery or external voltage supply. The device also contains means for storing and distributing the materials for RNA synthesis.

[00654] Optionally, the sample block contains a module for separating the synthesized nucleic acids. Alternatively, the device contains a separation module operably linked to the sample block. Preferably the device contains a means for analysis of the synthesized nucleic acid. Such analysis includes sequence identity (demonstrated such as by hybridization), absence of non-desired sequences, measurement of integrity of synthesized mRNA (such has by microfluidic viscometry combined with spectrophotometry), and concentration and/or potency of modified RNA (such as by spectrophotometry).

[00655] In certain embodiments, the device is combined with a means for detection of pathogens present in a biological material obtained from a subject, e.g., the IBIS PLEX-ID system (Abbott, Abbott Park, IL) for microbial identification.

[00656] Suitable devices for use in delivering intradermal pharmaceutical compositions described herein include short needle devices such as those described in U.S. Patents 4,886,499; 5,190,521; 5,328,483; 5,527,288; 4,270,537; 5,015,235; 5,141,496; and 5,417,662. Intradermal compositions may be administered by devices which limit the effective penetration length of a needle into the skin, such as those described in PCT publication WO 99/34850 and functional equivalents thereof. Jet injection devices which deliver liquid compositions to the dermis via a liquid jet injector and/or via a needle which pierces the stratum corneum and produces a jet which reaches the dermis are

suitable. Jet injection devices are described, for example, in U.S. Patents 5,480,381; 5,599,302; 5,334,144; 5,993,412; 5,649,912; 5,569,189; 5,704,91 1; 5,383,851; 5,893,397; 5,466,220; 5,339,163; 5,312,335; 5,503,627; 5,064,413; 5,520,639; 4,596,556; 4,790,824; 4,941,880; 4,940,460; and PCT publications WO 97/37705 and WO 97/13537. Ballistic powder/particle delivery devices which use compressed gas to accelerate vaccine in powder form through the outer layers of the skin to the dermis are suitable. Alternatively or additionally, conventional syringes may be used in the classical mantoux method of intradermal administration.

[00657] In some embodiments, the device may be a pump or comprise a catheter for administration of compounds or compositions of the invention across the blood brain barrier. Such devices include but are not limited to a pressurized olfactory delivery device, iontophoresis devices, multi-layered microfluidic devices, and the like. Such devices may be portable or stationary. They may be implantable or externally tethered to the body or combinations thereof.

[00658] Devices for administration may be employed to deliver the signal-sensor polynucleotides, primary constructs or mmRNA of the present invention according to single, multi- or split-dosing regimens taught herein. Such devices are described below.

[00659] Method and devices known in the art for multi-administration to cells, organs and tissues are contemplated for use in conjunction with the methods and compositions disclosed herein as embodiments of the present invention. These include, for example, those methods and devices having multiple needles, hybrid devices employing for example lumens or catheters as well as devices utilizing heat, electric current or radiation driven mechanisms.

[00660] According to the present invention, these multi-administration devices may be utilized to deliver the single, multi- or split doses contemplated herein.

[00661] A method for delivering therapeutic agents to a solid tissue has been described by Bahrami et al. and is taught for example in US Patent Publication 201 10230839, the contents of which are incorporated herein by reference in their entirety. According to Bahrami, an array of needles is incorporated into a device which delivers a substantially equal amount of fluid at any location in said solid tissue along each needle's length.

[00662] A device for delivery of biological material across the biological tissue has been described by Kodgule et al. and is taught for example in US Patent Publication 201 10172610, the contents of which are incorporated herein by reference in their entirety. According to Kodgule, multiple hollow micro-needles made of one or more metals and having outer diameters from about 200 microns to about 350 microns and lengths of at least 100 microns are incorporated into the device which delivers peptides, proteins, carbohydrates, nucleic acid molecules, lipids and other pharmaceutically active ingredients or combinations thereof.

[00663] A delivery probe for delivering a therapeutic agent to a tissue has been described by Gunday et al. and is taught for example in US Patent Publication 201 10270184, the contents of which are incorporated herein by reference in their entirety. According to Gunday, multiple needles are incorporated into the device which moves the attached capsules between an activated position and an inactivated position to force the agent out of the capsules through the needles.

[00664] A multiple-injection medical apparatus has been described by Assaf and is taught for example in US Patent Publication 201 10218497, the contents of which are incorporated herein by reference in their entirety. According to Assaf, multiple needles are incorporated into the device which has a chamber connected to one or more of said needles and a means for continuously refilling the chamber with the medical fluid after each injection.

[00665] In one embodiment, the signal-sensor polynucleotide, primary construct, or mmRNA is administered subcutaneously or intramuscularly via at least 3 needles to three different, optionally adjacent, sites simultaneously, or within a 60 minutes period (e.g., administration to 4, 5, 6, 7, 8, 9, or 10 sites simultaneously or within a 60 minute period). The split doses can be administered simultaneously to adjacent tissue using the devices described in U.S. Patent Publication Nos. 201 10230839 and 201 10218497, each of which is incorporated herein by reference.

[00666] An at least partially implantable system for injecting a substance into a patient's body, in particular a penis erection stimulation system has been described by Forsell and is taught for example in US Patent Publication 201 10196198, the contents of which are incorporated herein by reference in their entirety. According to Forsell,

multiple needles are incorporated into the device which is implanted along with one or more housings adjacent the patient's left and right corpora cavernosa. A reservoir and a pump are also implanted to supply drugs through the needles.

[00667] A method for the transdermal delivery of a therapeutic effective amount of iron has been described by Berenson and is taught for example in US Patent Publication 20100130910, the contents of which are incorporated herein by reference in their entirety. According to Berenson, multiple needles may be used to create multiple micro channels in stratum corneum to enhance transdermal delivery of the ionic iron on an iontophoretic patch.

[00668] A method for delivery of biological material across the biological tissue has been described by Kodgule et al and is taught for example in US Patent Publication 201 10196308, the contents of which are incorporated herein by reference in their entirety. According to Kodgule, multiple biodegradable microneedles containing a therapeutic active ingredient are incorporated in a device which delivers proteins, carbohydrates, nucleic acid molecules, lipids and other pharmaceutically active ingredients or combinations thereof.

[00669] A transdermal patch comprising a botulinum toxin composition has been described by Donovan and is taught for example in US Patent Publication 20080220020, the contents of which are incorporated herein by reference in their entirety. According to Donovan, multiple needles are incorporated into the patch which delivers botulinum toxin under stratum corneum through said needles which project through the stratum corneum of the skin without rupturing a blood vessel.

[00670] A small, disposable drug reservoir, or patch pump, which can hold approximately 0.2 to 15 mL of liquid formulations can be placed on the skin and deliver the formulation continuously subcutaneously using a small bore needed (e.g., 26 to 34 gauge). As non-limiting examples, the patch pump may be 50 mm by 76 mm by 20 mm spring loaded having a 30 to 34 gauge needle (BD™ Microinfuser, Franklin Lakes NJ), 4 1 mm by 62 mm by 17 mm with a 2 mL reservoir used for drug delivery such as insulin (OMNIPOD®, Insulet Corporation Bedford, MA), or 43-60 mm diameter, 10 mm thick with a 0.5 to 10 mL reservoir (PATCHPUMP®, SteadyMed Therapeutics, San Francisco, CA). Further, the patch pump may be battery powered and/or rechargeable.

[00671] A cryoprobe for administration of an active agent to a location of cryogenic treatment has been described by Toubia and is taught for example in US Patent Publication 20080140061, the contents of which are incorporated herein by reference in their entirety. According to Toubia, multiple needles are incorporated into the probe which receives the active agent into a chamber and administers the agent to the tissue.

[00672] A method for treating or preventing inflammation or promoting healthy joints has been described by Stock et al and is taught for example in US Patent Publication 20090155186, the contents of which are incorporated herein by reference in their entirety. According to Stock, multiple needles are incorporated in a device which administers compositions containing signal transduction modulator compounds.

[00673] A multi-site injection system has been described by Kimmell et al. and is taught for example in US Patent Publication 20100256594, the contents of which are incorporated herein by reference in their entirety. According to Kimmell, multiple needles are incorporated into a device which delivers a medication into a stratum corneum through the needles.

[00674] A method for delivering interferons to the intradermal compartment has been described by Dekker et al. and is taught for example in US Patent Publication 20050181033, the contents of which are incorporated herein by reference in their entirety. According to Dekker, multiple needles having an outlet with an exposed height between 0 and 1 mm are incorporated into a device which improves pharmacokinetics and bioavailability by delivering the substance at a depth between 0.3 mm and 2 mm.

[00675] A method for delivering genes, enzymes and biological agents to tissue cells has described by Desai and is taught for example in US Patent Publication 20030073908, the contents of which are incorporated herein by reference in their entirety. According to Desai, multiple needles are incorporated into a device which is inserted into a body and delivers a medication fluid through said needles.

[00676] A method for treating cardiac arrhythmias with fibroblast cells has been described by Lee et al and is taught for example in US Patent Publication 20040005295, the contents of which are incorporated herein by reference in their entirety. According to Lee, multiple needles are incorporated into the device which delivers fibroblast cells into the local region of the tissue.

[00677] A method using a magnetically controlled pump for treating a brain tumor has been described by Shachar et al. and is taught for example in US Patent 7799012 (method) and 7799016 (device), the contents of which are incorporated herein by reference in their entirety. According Shachar, multiple needles were incorporated into the pump which pushes a medicating agent through the needles at a controlled rate.

[00678] Methods of treating functional disorders of the bladder in mammalian females have been described by Versi et al. and are taught for example in US Patent 8,029,496, the contents of which are incorporated herein by reference in their entirety. According to Versi, an array of micro-needles is incorporated into a device which delivers a therapeutic agent through the needles directly into the trigone of the bladder.

[00679] A micro-needle transdermal transport device has been described by Angel et al and is taught for example in US Patent 7,364,568, the contents of which are incorporated herein by reference in their entirety. According to Angel, multiple needles are incorporated into the device which transports a substance into a body surface through the needles which are inserted into the surface from different directions. The micro-needle transdermal transport device may be a solid micro-needle system or a hollow micro-needle system. As a non-limiting example, the solid micro-needle system may have up to a 0.5 mg capacity, with 300-1500 solid micro-needles per cm^2 about 150-700 μιη tall coated with a drug. The micro-needles penetrate the stratum corneum and remain in the skin for short duration (e.g., 20 seconds to 15 minutes). In another example, the hollow micro-needle system has up to a 3 mL capacity to deliver liquid formulations using 15-20 microneedles per cm2 being approximately 950 μιη tall. The micro-needles penetrate the skin to allow the liquid formulations to flow from the device into the skin. The hollow micro-needle system may be worn from 1 to 30 minutes depending on the formulation volume and viscocity.

[00680] A device for subcutaneous infusion has been described by Dalton et al and is taught for example in US Patent 7,150,726, the contents of which are incorporated herein by reference in their entirety. According to Dalton, multiple needles are incorporated into the device which delivers fluid through the needles into a subcutaneous tissue.

[00681] A device and a method for intradermal delivery of vaccines and gene therapeutic agents through microcannula have been described by Mikszta et al. and are

taught for example in US Patent 7,473,247, the contents of which are incorporated herein by reference in their entirety. According to Mitszta, at least one hollow micro-needle is incorporated into the device which delivers the vaccines to the subject's skin to a depth of between 0.025 mm and 2 mm.

[00682] A method of delivering insulin has been described by Pettis et al and is taught for example in US Patent 7,722,595, the contents of which are incorporated herein by reference in their entirety. According to Pettis, two needles are incorporated into a device wherein both needles insert essentially simultaneously into the skin with the first at a depth of less than 2.5 mm to deliver insulin to intradermal compartment and the second at a depth of greater than 2.5 mm and less than 5.0 mm to deliver insulin to subcutaneous compartment.

[00683] Cutaneous injection delivery under suction has been described by Kochamba et al. and is taught for example in US Patent 6,896,666, the contents of which are incorporated herein by reference in their entirety. According to Kochamba, multiple needles in relative adjacency with each other are incorporated into a device which injects a fluid below the cutaneous layer.

[00684] A device for withdrawing or delivering a substance through the skin has been described by Down et al and is taught for example in US Patent 6,607,513, the contents of which are incorporated herein by reference in their entirety. According to Down, multiple skin penetrating members which are incorporated into the device have lengths of about 100 microns to about 2000 microns and are about 30 to 50 gauge.

[00685] A device for delivering a substance to the skin has been described by Palmer et al and is taught for example in US Patent 6,537,242, the contents of which are incorporated herein by reference in their entirety. According to Palmer, an array of micro-needles is incorporated into the device which uses a stretching assembly to enhance the contact of the needles with the skin and provides a more uniform delivery of the substance.

[00686] A perfusion device for localized drug delivery has been described by Zamoyski and is taught for example in US Patent 6,468,247, the contents of which are incorporated herein by reference in their entirety. According to Zamoyski, multiple hypodermic

needles are incorporated into the device which injects the contents of the hypodermics into a tissue as said hypodermics are being retracted.

[00687] A method for enhanced transport of drugs and biological molecules across tissue by improving the interaction between micro-needles and human skin has been described by Prausnitz et al. and is taught for example in US Patent 6,743,21 1, the contents of which are incorporated herein by reference in their entirety. According to Prausnitz, multiple micro-needles are incorporated into a device which is able to present a more rigid and less deformable surface to which the micro-needles are applied.

[00688] A device for intraorgan administration of medicinal agents has been described by Ting et al and is taught for example in US Patent 6,077,251, the contents of which are incorporated herein by reference in their entirety. According to Ting, multiple needles having side openings for enhanced administration are incorporated into a device which by extending and retracting said needles from and into the needle chamber forces a medicinal agent from a reservoir into said needles and injects said medicinal agent into a target organ.

[00689] A multiple needle holder and a subcutaneous multiple channel infusion port has been described by Brown and is taught for example in US Patent 4,695,273, the contents of which are incorporated herein by reference in their entirety. According to Brown, multiple needles on the needle holder are inserted through the septum of the infusion port and communicate with isolated chambers in said infusion port.

[00690] A dual hypodermic syringe has been described by Horn and is taught for example in US Patent 3,552,394, the contents of which are incorporated herein by reference in their entirety. According to Horn, two needles incorporated into the device are spaced apart less than 68 mm and may be of different styles and lengths, thus enabling injections to be made to different depths.

[00691] A syringe with multiple needles and multiple fluid compartments has been described by Hershberg and is taught for example in US Patent 3,572,336, the contents of which are incorporated herein by reference in their entirety. According to Hershberg, multiple needles are incorporated into the syringe which has multiple fluid compartments and is capable of simultaneously administering incompatible drugs which are not able to be mixed for one injection.

[00692] A surgical instrument for intradermal injection of fluids has been described by Eliscu et al. and is taught for example in US Patent 2,588,623, the contents of which are incorporated herein by reference in their entirety. According to Eliscu, multiple needles are incorporated into the instrument which injects fluids intradermally with a wider disperse.

[00693] An apparatus for simultaneous delivery of a substance to multiple breast milk ducts has been described by Hung and is taught for example in EP 1818017, the contents of which are incorporated herein by reference in their entirety. According to Hung, multiple lumens are incorporated into the device which inserts though the orifices of the ductal networks and delivers a fluid to the ductal networks.

[00694] A catheter for introduction of medications to the tissue of a heart or other organs has been described by Tkebuchava and is taught for example in WO2006138109, the contents of which are incorporated herein by reference in their entirety. According to Tkebuchava, two curved needles are incorporated which enter the organ wall in a flattened trajectory.

[00695] Devices for delivering medical agents have been described by Mckay et al. and are taught for example in WO20061 18804, the content of which are incorporated herein by reference in their entirety. According to Mckay, multiple needles with multiple orifices on each needle are incorporated into the devices to facilitate regional delivery to a tissue, such as the interior disc space of a spinal disc.

[00696] A method for directly delivering an immunomodulatory substance into an intradermal space within a mammalian skin has been described by Pettis and is taught for example in WO2004020014, the contents of which are incorporated herein by reference in their entirety. According to Pettis, multiple needles are incorporated into a device which delivers the substance through the needles to a depth between 0.3 mm and 2 mm.

[00697] Methods and devices for administration of substances into at least two compartments in skin for systemic absorption and improved pharmacokinetics have been described by Pettis et al. and are taught for example in WO2003094995, the contents of which are incorporated herein by reference in their entirety. According to Pettis, multiple needles having lengths between about 300 μιη and about 5 mm are incorporated into a

device which delivers to intradermal and subcutaneous tissue compartments simultaneously.

[00698] A drug delivery device with needles and a roller has been described by Zimmerman et al. and is taught for example in WO2012006259, the contents of which are incorporated herein by reference in their entirety. According to Zimmerman, multiple hollow needles positioned in a roller are incorporated into the device which delivers the content in a reservoir through the needles as the roller rotates.

Methods and Devices utilizing catheters and/or lumens

[00699] Methods and devices using catheters and lumens may be employed to administer the mmRNA of the present invention on a single, multi- or split dosing schedule. Such methods and devices are described below.

[00700] A catheter-based delivery of skeletal myoblasts to the myocardium of damaged hearts has been described by Jacoby et al and is taught for example in US Patent Publication 20060263338, the contents of which are incorporated herein by reference in their entirety. According to Jacoby, multiple needles are incorporated into the device at least part of which is inserted into a blood vessel and delivers the cell composition through the needles into the localized region of the subject's heart.

[00701] An apparatus for treating asthma using neurotoxin has been described by Deem et al and is taught for example in US Patent Publication 20060225742, the contents of which are incorporated herein by reference in their entirety. According to Deem, multiple needles are incorporated into the device which delivers neurotoxin through the needles into the bronchial tissue.

[00702] A method for administering multiple-component therapies has been described by Nayak and is taught for example in US Patent 7,699,803, the contents of which are incorporated herein by reference in their entirety. According to Nayak, multiple injection cannulas may be incorporated into a device wherein depth slots may be included for controlling the depth at which the therapeutic substance is delivered within the tissue.

[00703] A surgical device for ablating a channel and delivering at least one therapeutic agent into a desired region of the tissue has been described by Mclntyre et al and is taught for example in US Patent 8,012,096, the contents of which are incorporated herein by reference in their entirety. According to Mclntyre, multiple needles are incorporated

into the device which dispenses a therapeutic agent into a region of tissue surrounding the channel and is particularly well suited for transmyocardial revascularization operations.

[00704] Methods of treating functional disorders of the bladder in mammalian females have been described by Versi et al and are taught for example in US Patent 8,029,496, the contents of which are incorporated herein by reference in their entirety. According to Versi, an array of micro-needles is incorporated into a device which delivers a therapeutic agent through the needles directly into the trigone of the bladder.

[00705] A device and a method for delivering fluid into a flexible biological barrier have been described by Yeshurun et al. and are taught for example in US Patent 7,998,119 (device) and 8,007,466 (method), the contents of which are incorporated herein by reference in their entirety. According to Yeshurun, the micro-needles on the device penetrate and extend into the flexible biological barrier and fluid is injected through the bore of the hollow micro-needles.

[00706] A method for epicardially injecting a substance into an area of tissue of a heart having an epicardial surface and disposed within a torso has been described by Bonner et al and is taught for example in US Patent 7,628,780, the contents of which are incorporated herein by reference in their entirety. According to Bonner, the devices have elongate shafts and distal injection heads for driving needles into tissue and injecting medical agents into the tissue through the needles.

[00707] A device for sealing a puncture has been described by Nielsen et al and is taught for example in US Patent 7,972,358, the contents of which are incorporated herein by reference in their entirety. According to Nielsen, multiple needles are incorporated into the device which delivers a closure agent into the tissue surrounding the puncture tract.

[00708] A method for myogenesis and angiogenesis has been described by Chiu et al. and is taught for example in US Patent 6,551,338, the contents of which are incorporated herein by reference in their entirety. According to Chiu, 5 to 15 needles having a maximum diameter of at least 1.25 mm and a length effective to provide a puncture depth of 6 to 20 mm are incorporated into a device which inserts into proximity with a myocardium and supplies an exogeneous angiogenic or myogenic factor to said myocardium through the conduits which are in at least some of said needles.

[00709] A method for the treatment of prostate tissue has been described by Bolmsj et al. and is taught for example in US Patent 6,524,270, the contents of which are incorporated herein by reference in their entirety. According to Bolmsj, a device comprising a catheter which is inserted through the urethra has at least one hollow tip extendible into the surrounding prostate tissue. An astringent and analgesic medicine is administered through said tip into said prostate tissue.

[00710] A method for infusing fluids to an intraosseous site has been described by Findlay et al. and is taught for example in US Patent 6,761,726, the contents of which are incorporated herein by reference in their entirety. According to Findlay, multiple needles are incorporated into a device which is capable of penetrating a hard shell of material covered by a layer of soft material and delivers a fluid at a predetermined distance below said hard shell of material.

[00711] A device for injecting medications into a vessel wall has been described by Vigil et al. and is taught for example in US Patent 5,713,863, the contents of which are incorporated herein by reference in their entirety. According to Vigil, multiple injectors are mounted on each of the flexible tubes in the device which introduces a medication fluid through a multi-lumen catheter, into said flexible tubes and out of said injectors for infusion into the vessel wall.

[00712] A catheter for delivering therapeutic and/or diagnostic agents to the tissue surrounding a bodily passageway has been described by Faxon et al. and is taught for example in US Patent 5,464,395, the contents of which are incorporated herein by reference in their entirety. According to Faxon, at least one needle cannula is incorporated into the catheter which delivers the desired agents to the tissue through said needles which project outboard of the catheter.

[00713] Balloon catheters for delivering therapeutic agents have been described by Orr and are taught for example in WO20 10024871, the contents of which are incorporated herein by reference in their entirety. According to Orr, multiple needles are incorporated into the devices which deliver the therapeutic agents to different depths within the tissue.

Methods and Devices utilizing electrical current

[00714] Methods and devices utilizing electric current may be employed to deliver the mmRNA of the present invention according to the single, multi- or split dosing regimens taught herein. Such methods and devices are described below.

[00715] An electro collagen induction therapy device has been described by Marquez and is taught for example in US Patent Publication 20090137945, the contents of which are incorporated herein by reference in their entirety. According to Marquez, multiple needles are incorporated into the device which repeatedly pierce the skin and draw in the skin a portion of the substance which is applied to the skin first.

[00716] An electrokinetic system has been described by Etheredge et al. and is taught for example in US Patent Publication 20070185432, the contents of which are incorporated herein by reference in their entirety. According to Etheredge, micro-needles are incorporated into a device which drives by an electrical current the medication through the needles into the targeted treatment site.

[00717] An iontophoresis device has been described by Matsumura et al. and is taught for example in US Patent 7,437,189, the contents of which are incorporated herein by reference in their entirety. According to Matsumura, multiple needles are incorporated into the device which is capable of delivering ionizable drug into a living body at higher speed or with higher efficiency.

[00718] Intradermal delivery of biologically active agents by needle-free injection and electroporation has been described by Hoffmann et al and is taught for example in US Patent 7,171,264, the contents of which are incorporated herein by reference in their entirety. According to Hoffmann, one or more needle-free injectors are incorporated into an electroporation device and the combination of needle-free injection and electroporation is sufficient to introduce the agent into cells in skin, muscle or mucosa.

[00719] A method for electropermeabilization-mediated intracellular delivery has been described by Lundkvist et al. and is taught for example in US Patent 6,625,486, the contents of which are incorporated herein by reference in their entirety. According to Lundkvist, a pair of needle electrodes is incorporated into a catheter. Said catheter is positioned into a body lumen followed by extending said needle electrodes to penetrate into the tissue surrounding said lumen. Then the device introduces an agent through at least one of said needle electrodes and applies electric field by said pair of needle

electrodes to allow said agent pass through the cell membranes into the cells at the treatment site.

[00720] A delivery system for transdermal immunization has been described by Levin et al. and is taught for example in WO2006003659, the contents of which are incorporated herein by reference in their entirety. According to Levin, multiple electrodes are incorporated into the device which applies electrical energy between the electrodes to generate micro channels in the skin to facilitate transdermal delivery.

[00721] A method for delivering RF energy into skin has been described by Schomacker and is taught for example in WO201 1163264, the contents of which are incorporated herein by reference in their entirety. According to Schomacker, multiple needles are incorporated into a device which applies vacuum to draw skin into contact with a plate so that needles insert into skin through the holes on the plate and deliver RF energy.

VII. Definitions

[00722] At various places in the present specification, substituents of compounds of the present disclosure are disclosed in groups or in ranges. It is specifically intended that the present disclosure include each and every individual subcombination of the members of such groups and ranges. For example, the term "C_{i_6} alkyl" is specifically intended to individually disclose methyl, ethyl, C_3 alkyl, C_4 alkyl, C_5 alkyl, and C_6 alkyl.

[00723] *About:* As used herein, the term "about" means +/- 10% of the recited value.

[00724] *Administered in combination:* As used herein, the term "administered in combination" or "combined administration" means that two or more agents are administered to a subject at the same time or within an interval such that there may be an overlap of an effect of each agent on the patient. In some embodiments, they are administered within about 60, 30, 15, 10, 5, or 1 minute of one another. In some embodiments, the administrations of the agents are spaced sufficiently closely together such that a combinatorial *{e.g.,* a synergistic) effect is achieved.

[00725] *Animal:* As used herein, the term "animal" refers to any member of the animal kingdom. In some embodiments, "animal" refers to humans at any stage of development. In some embodiments, "animal" refers to non-human animals at any stage of development. In certain embodiments, the non-human animal is a mammal *{e.g.,* a

rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, and worms. In some embodiments, the animal is a transgenic animal, genetically-engineered animal, or a clone.

[00726] *Antigens of interest or desired antigens:* As used herein, the terms "antigens of interest" or "desired antigens" include those proteins and other biomolecules provided herein that are immunospecifically bound by the antibodies and fragments, mutants, variants, and alterations thereof described herein. Examples of antigens of interest include, but are not limited to, insulin, insulin-like growth factor, hGH, tPA, cytokines, such as interleukins (IL), e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-1 1, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, interferon (IFN) alpha, IFN beta, IFN gamma, IFN omega or IFN tau, tumor necrosis factor (TNF), such as TNF alpha and TNF beta, TNF gamma, TRAIL; G-CSF, GM-CSF, M-CSF, MCP-1 and VEGF.

[00727] *Approximately:* As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[00728] *Associated with:* As used herein, the terms "associated with," "conjugated," "linked," "attached," and "tethered," when used with respect to two or more moieties, means that the moieties are physically associated or connected with one another, either directly or via one or more additional moieties that serves as a linking agent, to form a structure that is sufficiently stable so that the moieties remain physically associated under the conditions in which the structure is used, *e.g.*, physiological conditions. An "association" need not be strictly through direct covalent chemical bonding. It may also suggest ionic or hydrogen bonding or a hybridization based connectivity sufficiently stable such that the "associated" entities remain physically associated.

[00729] *Bifunctional:* As used herein, the term "bifunctional" refers to any substance, molecule or moiety which is capable of or maintains at least two functions. The functions may effect the same outcome or a different outcome. The structure that produces the function may be the same or different. For example, bifunctional modified RNAs of the present invention may encode a cytotoxic peptide (a first function) while those nucleosides which comprise the encoding RNA are, in and of themselves, cytotoxic (second function). In this example, delivery of the bifunctional modified RNA to a cancer cell would produce not only a peptide or protein molecule which may ameliorate or treat the cancer but would also deliver a cytotoxic payload of nucleosides to the cell should degradation, instead of translation of the modified RNA, occur.

[00730] *Biocompatible:* As used herein, the term "biocompatible" means compatible with living cells, tissues, organs or systems posing little to no risk of injury, toxicity or rejection by the immune system.

[00731] *Biodegradable:* As used herein, the term "biodegradable" means capable of being broken down into innocuous products by the action of living things.

[00732] *Biologically active:* As used herein, the phrase "biologically active" refers to a characteristic of any substance that has activity in a biological system and/or organism. For instance, a substance that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active. In particular embodiments, signal-sensor polynucleotide, primary construct or mmRNA of the present invention may be considered biologically active if even a portion of the signal-sensor polynucleotide, primary construct or mmRNA is biologically active or mimics an activity considered biologically relevant.

[00733] *Cancer:* As used herein, the term "cancer" in a subject refers to the presence of cells possessing characteristics, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. Often, cancer cells will be in the form of a tumor, but such cells may exist alone within a subject, or may circulate in the blood stream as independent cells, such as leukemic cells.

[00734] *Cell growth:* As used herein, the term "cell growth" is principally associated with growth in cell numbers, which occurs by means of cell reproduction (i.e.

proliferation) when the rate of the latter is greater than the rate of cell death (e.g. by apoptosis or necrosis).

[00735] *Chemical terms:* The following provides the definition of various chemical terms from "acyl" to "thiol."

[00736] The term "acyl," as used herein, represents a hydrogen or an alkyl group (e.g., a haloalkyl group), as defined herein, that is attached to the parent molecular group through a carbonyl group, as defined herein, and is exemplified by formyl (i.e., a carboxyaldehyde group), acetyl, propionyl, butanoyl and the like. Exemplary unsubstituted acyl groups include from 1 to 7, from 1 to 11, or from 1 to 21 carbons. In some embodiments, the alkyl group is further substituted with 1, 2, 3, or 4 substituents as described herein.

[00737] The term "acylamino," as used herein, represents an acyl group, as defined herein, attached to the parent molecular group though an amino group, as defined herein (i.e., -N(R^{N1})-C(0)-R, where R is H or an optionally substituted C_{1-6}, C_{1-10}, or C_{i-20} alkyl group and R^{N1} is as defined herein). Exemplary unsubstituted acylamino groups include from 1 to 41 carbons (e.g., from 1 to 7, from 1 to 13, from 1 to 21, from 2 to 7, from 2 to 13, from 2 to 21, or from 2 to 41 carbons). In some embodiments, the alkyl group is further substituted with 1, 2, 3, or 4 substituents as described herein, and/or the amino group is -NH_2 or -NHR^{N1}, wherein R^{N1} is, independently, OH, NO_2, NH_2, NR^{N2}_2, SO_2OR^{N2}, SO_2R^{N2}, SOR^{N2}, alkyl, or aryl, and each R^{N2} can be H, alkyl, or aryl.

[00738] The term "acyloxy," as used herein, represents an acyl group, as defined herein, attached to the parent molecular group though an oxygen atom (i.e., -0-C(0)-R, where R is H or an optionally substituted C_{i-6}, C_{1-10}, or C_{i-20} alkyl group). Exemplary unsubstituted acyloxy groups include from 1 to 21 carbons (e.g., from 1 to 7 or from 1 to 11 carbons). In some embodiments, the alkyl group is further substituted with 1, 2, 3, or 4 substituents as described herein, and/or the amino group is -NH_2 or -NHR^{N1}, wherein R^{N1} is, independently, OH, NO_2, NH_2, NR^{N2}_2, SO_2OR^{N2}, SO_2R^{N2}, SOR^{N2}, alkyl, or aryl, and each R^{N2} can be H, alkyl, or aryl.

[00739] The term "alkaryl," as used herein, represents an aryl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein. Exemplary unsubstituted alkaryl groups are from 7 to 30 carbons (e.g., from 7 to 16 or

from 7 to 20 carbons, such as **Ci–6 alk-C6-io** aryl, **C1-10 alk-C6-io** aryl, or **Ci–2o alk-C6-io** aryl). In some embodiments, the alkylene and the aryl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective groups. Other groups preceded by the prefix "alk-" are defined in the same manner, where "alk" refers to a **Ci-6** alkylene, unless otherwise noted, and the attached chemical structure is as defined herein.

[00740] The term "alkcycloalkyl" represents a cycloalkyl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein (e.g., an alkylene group of from 1 to 4, from 1 to 6, from 1 to 10, or form 1 to 20 carbons). In some embodiments, the alkylene and the cycloalkyl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective group.

[00741] The term "alkenyl," as used herein, represents monovalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds and is exemplified by ethenyl, 1-propenyl, 2-propenyl, 2-methyl-l-propenyl, 1-butenyl, 2-butenyl, and the like. Alkenyls include both cis and trans isomers. Alkenyl groups may be optionally substituted with 1, 2, 3, or 4 substituent groups that are selected, independently, from amino, aryl, cycloalkyl, or heterocyclyl (e.g., heteroaryl), as defined herein, or any of the exemplary alkyl substituent groups described herein.

[00742] The term "alkenyloxy" represents a chemical substituent of formula -OR, where R is a **C2-2o** alkenyl group (e.g., **C2-6** or **C2–io** alkenyl), unless otherwise specified. Exemplary alkenyloxy groups include ethenyloxy, propenyloxy, and the like. In some embodiments, the alkenyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein (e.g., a hydroxy group).

[00743] The term "alkheteroaryl" refers to a heteroaryl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein. Exemplary unsubstituted alkheteroaryl groups are from 2 to 32 carbons (e.g., from 2 to 22, from 2 to 18, from 2 to 17, from 2 to 16, from 3 to 15, from 2 to 14, from 2 to 13, or from 2 to 12 carbons, such as **Ci–6 alk-Ci-i2** heteroaryl, **C1-10 alk-Ci-i2** heteroaryl, or **Ci–2o alk-Ci-i2** heteroaryl). In some embodiments, the alkylene and the heteroaryl each can be

further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective group. Alkheteroaryl groups are a subset of alkheterocyclyl groups.

[00744] The term "alkheterocyclyl" represents a heterocyclyl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein. Exemplary unsubstituted alkheterocyclyl groups are from 2 to 32 carbons (e.g., from 2 to 22, from 2 to 18, from 2 to 17, from 2 to 16, from 3 to 15, from 2 to 14, from 2 to 13, or from 2 to 12 carbons, such as **Ci_6 alk-Ci_i2** heterocyclyl, **C1-10 alk-Ci_i2** heterocyclyl, or **Ci_2o alk-Ci_i2** heterocyclyl). In some embodiments, the alkylene and the heterocyclyl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective group.

[00745] The term "alkoxy" represents a chemical substituent of formula -OR, where R is a **Ci_2o** alkyl group (e.g., **Ci_6** or **C1-10** alkyl), unless otherwise specified. Exemplary alkoxy groups include methoxy, ethoxy, propoxy (e.g., n-propoxy and isopropoxy), t-butoxy, and the like. In some embodiments, the alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein (e.g., hydroxy or alkoxy).

[00746] The term "alkoxyalkoxy" represents an alkoxy group that is substituted with an alkoxy group. Exemplary unsubstituted alkoxyalkoxy groups include between 2 to 40 carbons (e.g., from 2 to 12 or from 2 to 20 carbons, such as **Ci_6 alkoxy-Ci_6** alkoxy, **C1-10 alkoxy-Ci_io** alkoxy, or **Ci_2o alkoxy-Ci_2o** alkoxy). In some embodiments, the each alkoxy group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00747] The term "alkoxyalkyl" represents an alkyl group that is substituted with an alkoxy group. Exemplary unsubstituted alkoxyalkyl groups include between 2 to 40 carbons (e.g., from 2 to 12 or from 2 to 20 carbons, such as **Ci_6 alkoxy-Ci_6** alkyl, **C1-10 alkoxy-Ci_io** alkyl, or **Ci_2o alkoxy-Ci_2o** alkyl). In some embodiments, the alkyl and the alkoxy each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective group.

[00748] The term "alkoxycarbonyl," as used herein, represents an alkoxy, as defined herein, attached to the parent molecular group through a carbonyl atom (e.g., -C(0)-OR, where R is H or an optionally substituted **Ci_6**, **C1-10**, or **Ci_2o** alkyl group). Exemplary unsubstituted alkoxycarbonyl include from 1 to 21 carbons (e.g., from 1 to 11 or from 1

to 7 carbons). In some embodiments, the alkoxy group is further substituted with 1, 2, 3, or 4 substituents as described herein.

[00749] The term "alkoxy carbonylalkoxy," as used herein, represents an alkoxy group, as defined herein, that is substituted with an alkoxycarbonyl group, as defined herein (e.g., -0-alkyl-C(0)-OR, where R is an optionally substituted C_{1-6}, C_{1-10}, or $Ci_{-2}o$ alkyl group). Exemplary unsubstituted alkoxycarbonylalkoxy include from 3 to 41 carbons (e.g., from 3 to 10, from 3 to 13, from 3 to 17, from 3 to 21, or from 3 to 31 carbons, such as Ci_{-6} alkoxycarbonyl-Ci_6 alkoxy, C_{1-10} alkoxycarbonyl-Ci_io alkoxy, or $Ci_{-2}o$ alkoxycarbonyl-$Ci_{-2}o$ alkoxy). In some embodiments, each alkoxy group is further independently substituted with 1, 2, 3, or 4 substituents, as described herein (e.g., a hydroxy group).

[00750] The term "alkoxycarbonylalkyl," as used herein, represents an alkyl group, as defined herein, that is substituted with an alkoxycarbonyl group, as defined herein (e.g., -alkyl-C(0)-OR, where R is an optionally substituted Ci_{-20}, C_{1-10}, or Ci_{-6} alkyl group). Exemplary unsubstituted alkoxycarbonylalkyl include from 3 to 41 carbons (e.g., from 3 to 10, from 3 to 13, from 3 to 17, from 3 to 21, or from 3 to 31 carbons, such as Ci_{-6} alkoxycarbonyl-Ci_6 alkyl, C_{1-10} alkoxycarbonyl-Ci_io alkyl, or $Ci_{-2}o$ alkoxycarbonyl-$Ci_{-2}o$ alkyl). In some embodiments, each alkyl and alkoxy group is further independently substituted with 1, 2, 3, or 4 substituents as described herein (e.g., a hydroxy group).

[00751] The term "alkyl," as used herein, is inclusive of both straight chain and branched chain saturated groups from 1 to 20 carbons (e.g., from 1 to 10 or from 1 to 6), unless otherwise specified. Alkyl groups are exemplified by methyl, ethyl, n- and iso-propyl, n-, sec-, iso- and tert-butyl, neopentyl, and the like, and may be optionally substituted with one, two, three, or, in the case of alkyl groups of two carbons or more, four substituents independently selected from the group consisting of: (1) Ci_{-6} alkoxy; (2) Ci_6 alkylsulfinyl; (3) amino, as defined herein (e.g., unsubstituted amino (i.e., $-NH_2$) or a substituted amino (i.e., $-N(R^{N1})_2$, where R^{N1} is as defined for amino); (4) C_{6-io} aryl-Ci_{-6} alkoxy; (5) azido; (6) halo; (7) (C_{2-9} heterocyclyl)oxy; (8) hydroxy; (9) nitro; (10) oxo (e.g., carboxyaldehyde or acyl); (11) Ci_{-7} spirocyclyl; (12) thioalkoxy; (13) thiol; (14) $-C0_2R^{A'}$, where R^A is selected from the group consisting of (a) $Ci_{-2}o$ alkyl (e.g., Ci_{-6} alkyl), (b) C_{2-20} alkenyl (e.g., C_{2-6} alkenyl), (c) C_{6-10} aryl, (d) hydrogen, (e) Ci_{-6} alk-C_{6-10}

aryl, (f) amino-C_{1-20} alkyl, (g) polyethylene glycol of -$(CH_2)_{s2}(OCH_2CH_2)_{si}(CH_2)_{s3}OR'$, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or C_{1-20} alkyl, and (h) amino-polyethylene glycol of -$NR^{N1}(CH_2)_{s2}(CH_2CH_20)_{si}(CH_2)_{s3}NR^{N1}$, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted C_{1-6} alkyl; (15) -C(0)$NR^{B'}R^{C'}$, where each of $R^{B'}$ and $R^{C'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) C_{1-6} alkyl, (c) C_{6-10} aryl, and (d) C_{1-6} alk-C_{6-10} aryl; (16) -$S0_2R^{D'}$, where $R^{D'}$ is selected from the group consisting of (a) C_{1-6} alkyl, (b) C_{6-10} aryl, (c) C_{1-6} alk-C_{6-10} aryl, and (d) hydroxy; (17) -$S0_2NR^{E'}R^{F'}$, where each of $R^{E'}$ and $R^{F'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) C_{1-6} alkyl, (c) C_{6-10} aryl and (d) C_{1-6} alk-C_{6-10} aryl; (18) -C(0)$R^{G'}$, where $R^{G'}$ is selected from the group consisting of (a) C_{1-20} alkyl (e.g., C_{1-6} alkyl), (b) C_{2-20} alkenyl (e.g., C_{2-6} alkenyl), (c) C_{6-10} aryl, (d) hydrogen, (e) C_{1-6} alk-C_{6-10} aryl, (f) amino-C_{1-20} alkyl, (g) polyethylene glycol of -$(CH_2)_{s2}(OCH_2CH_2)_{si}(CH_2)_{s3}OR'$, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or C_{1-20} alkyl, and (h) amino-polyethylene glycol of -$NR^{N1}(CH_2)_{s2}(CH_2CH_20)_{si}(CH_2)_{s3}NR^{N1}$, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted C_{1-6} alkyl; (19) -$NR^{H'}C(0)R^{I'}$, wherein $R^{H'}$ is selected from the group consisting of (al) hydrogen and (bl) C_{1-6} alkyl, and $R^{I'}$ is selected from the group consisting of (a2) C_{1-20} alkyl (e.g., C_{1-6} alkyl), (b2) C_{2-20} alkenyl (e.g., C_{2-6} alkenyl), (c2) C_{6-10} aryl, (d2) hydrogen, (e2) C_{1-6} alk-C_{6-10} aryl, (f2) amino-C_{1-20} alkyl, (g2) polyethylene glycol of -$(CH_2)_{s2}(OCH_2CH_2)_{si}(CH_2)_{s3}OR'$, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or C_{1-20} alkyl, and (h2) amino-polyethylene glycol of -

$NR^{N1}(CH_2)_{s2}(CH_2CH_20)_si(CH_2)_{s3}NR^{N1}$, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted **Ci_6** alkyl; (20) **-NR$^{J'}$C(0)OR$^{K'}$,** wherein $R^{J'}$ is selected from the group consisting of (al) hydrogen and (bl) **Ci_6** alkyl, and $R^{K'}$ is selected from the group consisting of (a2) **Ci_20** alkyl (e.g., **Ci_6** alkyl), (b2) **C_{2-20}** alkenyl (e.g., **C_{2-6}** alkenyl), (c2) **C_{6-10}** aryl, (d2) hydrogen, (e2) **Ci_6 alk-C_{6-io}** aryl, (f2) amino **-Ci_20** alkyl, (g2) polyethylene glycol of $-(CH_2)_{s2}(OCH_2CH_2)si(CH_2)_S30R'$, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is **H** or **Ci_20** alkyl, and (h2) amino-polyethylene glycol of **-NR^{N1}(CH$_2$)$_{s2}$(CH$_2$CH$_2$0)$_s$i(CH$_2$)$_{s3}$**NR^{N1}, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted **Ci_6** alkyl; and (21) amidine. In some embodiments, each of these groups can be further substituted as described herein. For example, the alkylene group of a **Ci**-alkaryl can be further substituted with an oxo group to afford the respective aryloyl substituent.

[00752] The term "alkylene" and the prefix "alk-," as used herein, represent a saturated divalent hydrocarbon group derived from a straight or branched chain saturated hydrocarbon by the removal of two hydrogen atoms, and is exemplified by methylene, ethylene, isopropylene, and the like. The term "**C_{x-y}** alkylene" and the prefix "**C_{x-y}** alk-" represent alkylene groups having between x and y carbons. Exemplary values for x are 1, 2, 3, 4, 5, and 6, and exemplary values for y are 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, or 20 (e.g., **Ci_6**, **Ci_io,** C2-20, C2-6, C2-10, or C2-20 alkylene). In some embodiments, the alkylene can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for an alkyl group.

[00753] The term "alkylsulfmyl," as used herein, represents an alkyl group attached to the parent molecular group through an **-S(O)-** group. Exemplary unsubstituted alkylsulfmyl groups are from 1 to 6, from 1 to 10, or from 1 to 20 carbons. In some

embodiments, the alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00754] The term "alkylsulfmylalkyl," as used herein, represents an alkyl group, as defined herein, substituted by an alkylsulfmyl group. Exemplary unsubstituted alkylsulfmylalkyl groups are from 2 to 12, from 2 to 20, or from 2 to 40 carbons. In some embodiments, each alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00755] The term "alkynyl," as used herein, represents monovalent straight or branched chain groups from 2 to 20 carbon atoms (e.g., from 2 to 4, from 2 to 6, or from 2 to 10 carbons) containing a carbon-carbon triple bond and is exemplified by ethynyl, 1-propynyl, and the like. Alkynyl groups may be optionally substituted with 1, 2, 3, or 4 substituent groups that are selected, independently, from aryl, cycloalkyl, or heterocyclyl (e.g., heteroaryl), as defined herein, or any of the exemplary alkyl substituent groups described herein.

[00756] The term "alkynyloxy" represents a chemical substituent of formula -OR, where R is a C_{2-20} alkynyl group (e.g., C_{2-6} or C_{2-10} alkynyl), unless otherwise specified. Exemplary alkynyloxy groups include ethynyloxy, propynyloxy, and the like. In some embodiments, the alkynyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein (e.g., a hydroxy group).

[00757] The term "amidine," as used herein, represents a -C(=NH)NH_2 group.

[00758] The term "amino," as used herein, represents -$N(R^{N1})_2$, wherein each R^{N1} is, independently, H, OH, NO_2, $N(R^{N2})_2$, SO_2OR^{N2}, SO_2R^{N2}, SOR^{N2}, an *N*-protecting group, alkyl, alkenyl, alkynyl, alkoxy, aryl, alkaryl, cycloalkyl, alkcycloalkyl, carboxyalkyl, sulfoalkyl, heterocyclyl (e.g., heteroaryl), or alkheterocyclyl (e.g., alkheteroaryl), wherein each of these recited R^{N1} groups can be optionally substituted, as defined herein for each group; or two R^{N1} combine to form a heterocyclyl or an *N*-protecting group, and wherein each R^{N2} is, independently, H, alkyl, or aryl. The amino groups of the invention can be an unsubstituted amino (i.e., -NH_2) or a substituted amino (i.e., -$N(R^{N1})_2$). In a preferred embodiment, amino is -NH_2 or -NHR^{N1}, wherein R^{N1} is, independently, OH, NO_2, NH_2, NR^{N2}_2, SO_2OR^{N2}, SO_2R^{N2}, SOR^{N2}, alkyl, carboxyalkyl, sulfoalkyl, or aryl, and each R^{N2} can be H, C_{1-20} alkyl (e.g., C_{1-6} alkyl), or C_{6-10} aryl.

[00759] The term "amino acid," as described herein, refers to a molecule having a side chain, an amino group, and an acid group (e.g., a carboxy group of-C0 $_2$H or a sulfo group of -SO $_3$H), wherein the amino acid is attached to the parent molecular group by the side chain, amino group, or acid group (e.g., the side chain). In some embodiments, the amino acid is attached to the parent molecular group by a carbonyl group, where the side chain or amino group is attached to the carbonyl group. Exemplary side chains include an optionally substituted alkyl, aryl, heterocyclyl, alkaryl, alkheterocyclyl, aminoalkyl, carbamoylalkyl, and carboxyalkyl. Exemplary amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, hydroxynorvaline, isoleucine, leucine, lysine, methionine, norvaline, ornithine, phenylalanine, proline, pyrrolysine, selenocysteine, serine, taurine, threonine, tryptophan, tyrosine, and valine. Amino acid groups may be optionally substituted with one, two, three, or, in the case of amino acid groups of two carbons or more, four substituents independently selected from the group consisting of: (1) **Ci_6** alkoxy; (2) **Ci_6** alkylsulfinyl; (3) amino, as defined herein (e.g., unsubstituted amino (i.e., -NH_2) or a substituted amino (i.e., **-N(R^{N1})$_2$,** where R^{N1} is as defined for amino); (4) **C_{6-10} aryl-Ci_6** alkoxy; (5) azido; (6) halo; (7) **(C_{2-9}** heterocyclyl)oxy; **(8)** hydroxy; (9) nitro; (10) oxo (e.g., carboxyaldehyde or acyl); (11) **Ci_7** spirocyclyl; (12) thioalkoxy; (13) thiol; (14) -**C0 $_2$$R^{A'}$**, where R^A is selected from the group consisting of (a) **Ci_20** alkyl (e.g., **Ci_6** alkyl), (b) **C_{2-20}** alkenyl (e.g., **C_{2-6}** alkenyl), (c) **C_{6-10}** aryl, (d) hydrogen, (e) **Ci_6 alk-C_{6-10}** aryl, (f) **amino-Ci_20** alkyl, (g) polyethylene glycol of **-(CH_2)$_{s2}$(OCH_2CH_2)$_s$i(CH_2)$_{s3}$OR',** wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and **R'** is **H** or **Ci_20** alkyl, and (h) amino-polyethylene glycol of -**NR^{N1}(CH_2)$_{s2}$(CH_2CH_20)si(CH_2)$_{s3}$$NR^{N1}$,** wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted **Ci_6** alkyl; (15) -**C(0)NR** $^{B'}$**$R^{C'}$**, where each of $R^{B'}$ and $R^{C'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) **Ci_6** alkyl, (c) **C_6-io** aryl, and (d) **Ci_6 alk-C_6-io** aryl; (16) **-S0 $_2$$R^{D'}$**, where $R^{D'}$ is selected from the group consisting of (a) **Ci_6** alkyl, (b) **C_{6-io}** aryl, (c) **Ci_6 alk-C_{6-io}** aryl,

and (d) hydroxy; (17) -S0 $_2$NR$^{E'}$R$^{F'}$, where each of R$^{E'}$ and R$^{F'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) Ci$_{-6}$ alkyl, (c) C$_{6-10}$ aryl and (d) Ci$_{-6}$ alk-C$_{6-10}$ aryl; (18) -C(0)R$^{G'}$, where R$^{G'}$ is selected from the group consisting of (a) Ci$_{-20}$ alkyl (e.g., Ci$_{-6}$ alkyl), (b) C$_{2-20}$ alkenyl (e.g., C$_{2-6}$ alkenyl), (c) C$_{6-10}$ aryl, (d) hydrogen, (e) Ci$_{-6}$ alk-C$_{6-io}$ aryl, (f) amino-Ci$_{-20}$ alkyl, (g) polyethylene glycol of -(CH$_2$)$_{s2}$(OCH$_2$CH$_2$)$_s$i(CH$_2$)$_{s3}$0R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci$_{-20}$ alkyl, and (h) amino-polyethylene glycol of -NR^{N1}(CH$_2$)$_{s2}$(CH$_2$CH$_2$0)$_s$i(CH$_2$)$_{s3}$NR^{N1}, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted Ci$_{-6}$ alkyl; (19) -NR$^{H'}$C(0)R$^{I'}$, wherein R$^{H'}$ is selected from the group consisting of (al) hydrogen and (bl) Ci$_{-6}$ alkyl, and R$^{I'}$ is selected from the group consisting of (a2) Ci$_{-20}$ alkyl (e.g., Ci$_{-6}$ alkyl), (b2) C$_{2-20}$ alkenyl (e.g., C$_{2-6}$ alkenyl), (c2) C$_{6-io}$ aryl, (d2) hydrogen, (e2) Ci$_{-6}$ alk-C$_{6-io}$ aryl, (f2) amino-Ci$_{-20}$ alkyl, (g2) polyethylene glycol of -(CH$_2$)$_{s2}$(OCH$_2$CH$_2$)$_s$i(CH$_2$)$_{s3}$OR', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci$_{-20}$ alkyl, and (h2) amino-polyethylene glycol of -NR^{N1}(CH$_2$)$_{s2}$(CH$_2$CH$_2$0)**si** (CH$_2$)$_{s3}$NR^{N1}, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted Ci$_{-6}$ alkyl; (20) -NR$^{J'}$C(0)OR$^{K'}$, wherein R$^{J'}$ is selected from the group consisting of (al) hydrogen and (bl) Ci$_{-6}$ alkyl, and R$^{K'}$ is selected from the group consisting of (a2) Ci$_{-20}$ alkyl (e.g., Ci$_{-6}$ alkyl), (b2) C$_{2-20}$ alkenyl (e.g., C$_{2-6}$ alkenyl), (c2) C$_{6-10}$ aryl, (d2) hydrogen, (e2) Ci$_{-6}$ alk-C$_{6-io}$ aryl, (f2) amino-Ci$_{-20}$ alkyl, (g2) polyethylene glycol of -(CH$_2$)$_{s2}$(OCH$_2$CH$_2$)$_s$i(CH$_2$)$_{s3}$OR', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci$_{-20}$ alkyl, and (h2) amino-polyethylene glycol of

-$NR^{N1}(CH_{2)s2}(CH_2CH_20)_si(CH_{2)s3NR}{}^{N1}$, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted C_{i-6} alkyl; and (21) amidine. In some embodiments, each of these groups can be further substituted as described herein.

[00760] The term "aminoalkoxy," as used herein, represents an alkoxy group, as defined herein, substituted by an amino group, as defined herein. The alkyl and amino each can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for the respective group (e.g., $CC"_2R^{A'}$, where $R^{A'}$ is selected from the group consisting of (a) C_{i-6} alkyl, (b) C_{6-10} aryl, (c) hydrogen, and (d) C_{i-6} alk-C_{6-io} aryl, e.g., carboxy).

[00761] The term "aminoalkyl," as used herein, represents an alkyl group, as defined herein, substituted by an amino group, as defined herein. The alkyl and amino each can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for the respective group (e.g., $C0_2R^{A'}$, where $R^{A'}$ is selected from the group consisting of (a) C_{i-6} alkyl, (b) C_{6-io} aryl, (c) hydrogen, and (d) C_{i-6} alk-C_{6-io} aryl, e.g., carboxy).

[00762] The term "aryl," as used herein, represents a mono-, bicyclic, or multicyclic carbocyclic ring system having one or two aromatic rings and is exemplified by phenyl, naphthyl, 1,2-dihydronaphthyl, 1,2,3,4-tetrahydronaphthyl, anthracenyl, phenanthrenyl, fluorenyl, indanyl, indenyl, and the like, and may be optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from the group consisting of: (1) C_{i-7} acyl (e.g., carboxyaldehyde); (2) C_{i-2o} alkyl (e.g., C_{i-6} alkyl, C_{i-6} alkoxy-C_{i-6} alkyl, C_{i-6} alkylsulfmyl-C_{i-6} alkyl, amino-C_{i-6} alkyl, azido-C_{i-6} alkyl, (carboxyaldehyde)-C_{i-6} alkyl, halo-C_{i-6} alkyl (e.g., perfluoroalkyl), hydroxy-C_{i-6} alkyl, nitro-C_{i-6} alkyl, or C_{i-6} thioalkoxy-C_{i-6} alkyl); (3) C_{i-2o} alkoxy (e.g., C_{i-6} alkoxy, such as perfluoroalkoxy); (4) C_{i-6} alkylsulfinyl; (5) C_{6-1o} aryl; (6) amino; (7) C_{i-6} alk-C_{6-io} aryl; (8) azido; (9) C3-8 cycloalkyl; (10) C_{i-6} alk-C_{3-8} cycloalkyl; (11) halo; (12) C_{1-12} heterocyclyl (e.g., C_{1-12} heteroaryl); (13) (C_{1-12} heterocyclyl)oxy; (14) hydroxy; (15) nitro; (16) C_{i-2o} thioalkoxy (e.g., C_{i-6} thioalkoxy); (17) -$(CH_2)_qC0_2R^{A'}$, where q is an integer from zero to four, and $R^{A'}$ is selected from the group consisting of (a) C_{i-6} alkyl, (b) C_{6-1o} aryl, (c) hydrogen, and (d) C_{i-6} alk-C_{6-io} aryl; (18) -$(CH_2)_qCONR^{B'}R^{C'}$, where q is an integer from zero to four and where $R^{B'}$ and $R^{C'}$ are independently selected from the group consisting of (a)

hydrogen, (b) Ci_6 alkyl, (c) C6_io aryl, and (d) Ci_6 alk-C 6-io aryl; (19) -(CH 2)qS0 2R^D', where q is an integer from zero to four and where R^D' is selected from the group consisting of (a) alkyl, (b) C6-io aryl, and (c) alk-C 6-io aryl; (20) -(CH 2)qS0 2NR^E'R^F', where q is an integer from zero to four and where each of R^E' and R^F' is, independently, selected from the group consisting of (a) hydrogen, (b) Ci_6 alkyl, (c) C6-io aryl, and (d) Ci_6 alk-C 6_io aryl; (21) thiol; (22) C6_io aryloxy; (23) C3-8 cycloalkoxy; (24) C6_io aryl-Ci_6 alkoxy; (25) Ci_6 alk-Ci_i 2 heterocyclyl (e.g., Ci_6 alk-Ci_i 2 heteroaryl); (26) C2-2o alkenyl; and (27) C2-2o alkynyl. In some embodiments, each of these groups can be further substituted as described herein. For example, the alkylene group of a Ci-alkaryl or a Ci-alkheterocyclyl can be further substituted with an oxo group to afford the respective aryloyl and (heterocyclyl)oyl substituent group.

[00763] The term "arylalkoxy," as used herein, represents an alkaryl group, as defined herein, attached to the parent molecular group through an oxygen atom. Exemplary unsubstituted alkoxyalkyl groups include from 7 to 30 carbons (e.g., from 7 to 16 or from 7 to 20 carbons, such as C6-io aryl-Ci_6 alkoxy, C6-io aryl-Ci_io alkoxy, or C6-io aryl-Ci_2o alkoxy). In some embodiments, the arylalkoxy group can be substituted with 1, 2, 3, or 4 substituents as defined herein

[00764] The term "aryloxy" represents a chemical substituent of formula -OR', where R' is an aryl group of 6 to 18 carbons, unless otherwise specified. In some embodiments, the aryl group can be substituted with 1, 2, 3, or 4 substituents as defined herein.

[00765] The term "aryloyl," as used herein, represents an aryl group, as defined herein, that is attached to the parent molecular group through a carbonyl group. Exemplary unsubstituted aryloyl groups are of 7 to 11 carbons. In some embodiments, the aryl group can be substituted with 1, 2, 3, or 4 substituents as defined herein.

[00766] The term "azido" represents an -N 3 group, which can also be represented as -N=N=N.

[00767] The term "bicyclic," as used herein, refer to a structure having two rings, which may be aromatic or non-aromatic. Bicyclic structures include spirocyclyl groups, as defined herein, and two rings that share one or more bridges, where such bridges can include one atom or a chain including two, three, or more atoms. Exemplary bicyclic groups include a bicyclic carbocyclyl group, where the first and second rings are

carbocyclyl groups, as defined herein; a bicyclic aryl groups, where the first and second rings are aryl groups, as defined herein; bicyclic heterocyclyl groups, where the first ring is a heterocyclyl group and the second ring is a carbocyclyl (e.g., aryl) or heterocyclyl (e.g., heteroaryl) group; and bicyclic heteroaryl groups, where the first ring is a heteroaryl group and the second ring is a carbocyclyl (e.g., aryl) or heterocyclyl (e.g., heteroaryl) group. In some embodiments, the bicyclic group can be substituted with 1, 2, 3, or 4 substituents as defined herein for cycloalkyl, heterocyclyl, and aryl groups.

[00768] The terms "carbocyclic" and "carbocyclyl," as used herein, refer to an optionally substituted C_{3-12} monocyclic, bicyclic, or tricyclic structure in which the rings, which may be aromatic or non-aromatic, are formed by carbon atoms. Carbocyclic structures include cycloalkyl, cycloalkenyl, and aryl groups.

[00769] The term "carbamoyl," as used herein, represents -C(0)-N(R^{N1})$_2$, where the meaning of each R^{N1} is found in the definition of "amino" provided herein.

[00770] The term "carbamoylalkyl," as used herein, represents an alkyl group, as defined herein, substituted by a carbamoyl group, as defined herein. The alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00771] The term "carbamyl," as used herein, refers to a carbamate group having the structure

-NR^{N1}C(=0)OR or -OC(=0)N(R^{N1})$_2$, where the meaning of each R^{N1} is found in the definition of "amino" provided herein, and R is alkyl, cycloalkyl , alkcycloalkyl, aryl, alkaryl, heterocyclyl (e.g., heteroaryl), or alkheterocyclyl (e.g., alkheteroaryl), as defined herein.

[00772] The term "carbonyl," as used herein, represents a C(O) group, which can also be represented as C=0.

[00773] The term "carboxyaldehyde" represents an acyl group having the structure -CHO.

[00774] The term "carboxy," as used herein, means -C0$_2$H.

[00775] The term "carboxyalkoxy," as used herein, represents an alkoxy group, as defined herein, substituted by a carboxy group, as defined herein. The alkoxy group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for the alkyl group.

[00776] The term "carboxyalkyl," as used herein, represents an alkyl group, as defined herein, substituted by a carboxy group, as defined herein. The alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00777] The term "cyano," as used herein, represents an **-CN** group.

[00778] The term "cycloalkoxy" represents a chemical substituent of formula **-OR,** where **R** is a C_{3-8} cycloalkyl group, as defined herein, unless otherwise specified. The cycloalkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein. Exemplary unsubstituted cycloalkoxy groups are from 3 to 8 carbons. In some embodiment, the cycloalkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00779] The term "cycloalkyl," as used herein represents a monovalent saturated or unsaturated non-aromatic cyclic hydrocarbon group from three to eight carbons, unless otherwise specified, and is exemplified by cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, bicyclo[2.2.1.]heptyl, and the like. When the cycloalkyl group includes one carbon-carbon double bond, the cycloalkyl group can be referred to as a "cycloalkenyl" group. Exemplary cycloalkenyl groups include cyclopentenyl, cyclohexenyl, and the like. The cycloalkyl groups of this invention can be optionally substituted with: (1) **Ci_7** acyl (e.g., carboxyaldehyde); (2) **Ci_20** alkyl (e.g., **Ci_6** alkyl, **Ci_6 alkoxy-Ci_6** alkyl, **Ci_6** alkylsulfmyl-**Ci_6** alkyl, **amino-Ci_6** alkyl, **azido-Ci_6** alkyl, (carboxyaldehyde)-**Ci_6** alkyl, **halo-Ci_6** alkyl (e.g., perfluoroalkyl), hydroxy-**Ci_6** alkyl, nitro-**Ci_6** alkyl, or **Ci_6**thioalkoxy-**Ci_6** alkyl); (3) **Ci_20** alkoxy (e.g., **Ci_6** alkoxy, such as perfluoroalkoxy); (4) **Ci_6** alkylsulfmyl; (5) C_{6-10} aryl; (6) amino; (7) **Ci_6 alk-C_{6-10}** aryl; (8) azido; (9) C_{3-8} cycloalkyl; (10) **Ci_6 alk-C_{3-8}** cycloalkyl; (11) halo; (12) C_{1-12} heterocyclyl (e.g., C_{1-12} heteroaryl); (13) (C_{1-12} heterocyclyl)oxy; (14) hydroxy; (15) nitro; (16) **Ci_20** thioalkoxy (e.g., **Ci_6** thioalkoxy); (17) **-$(CH_2)_q C0_2 R^{A'}$**, where q is an integer from zero to four, and $R^{A'}$ is selected from the group consisting of (a) **Ci_6** alkyl, (b) C_{6-10} aryl, (c) hydrogen, and (d) **Ci_6 alk-C_{6-10}** aryl; (18) **-$(CH_2)_q CONR^{B'}R^{C'}$,** where q is an integer from zero to four and where $R^{B'}$ and $R^{C'}$ are independently selected from the group consisting of (a) hydrogen, (b) C_{6-10} alkyl, (c) C_{6-10} aryl, and (d) **Ci_6 alk-C_{6-10}** aryl; (19) **-$(CH_2)_q S0_2 R^{D'}$**, where q is an integer from zero to four and where $R^{D'}$ is selected from the group consisting of (a) C_{6-10} alkyl, (b) C_{6-10} aryl, and (c) **Ci_6 alk-C_{6-10}** aryl; (20)

-(CH $_2$)$_q$S0 $_2$NR$^{E'}$R$^{F'}$, where q is an integer from zero to four and where each of R$^{E'}$ and R$^{F'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) C_{6_io} alkyl, (c) C_{6}-io aryl, and (d) $Ci_{\_6}$ alk-C_{6}-io aryl; (21) thiol; (22) C_{6-10} aryloxy; (23) C_{3-8} cycloalkoxy; (24) C_{6}-io aryl-$Ci_{\_6}$ alkoxy; (25) $Ci_{\_6}$ alk-$Ci_{\_i2}$ heterocyclyl (e.g., $Ci_{\_6}$ alk-$Ci_{\_12}$ heteroaryl); (26) oxo; (27) C_{2-20} alkenyl; and (28) C_{2-20} alkynyl. In some embodiments, each of these groups can be further substituted as described herein. For example, the alkylene group of a Ci-alkaryl or a Ci-alkheterocyclyl can be further substituted with an oxo group to afford the respective aryloyl and (heterocyclyl)oyl substituent group.

[00780] The term "diastereomer," as used herein means stereoisomers that are not mirror images of one another and are non-superimposable on one another.

[00781] The term "effective amount" of an agent, as used herein, is that amount sufficient to effect beneficial or desired results, for example, clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. For example, in the context of administering an agent that treats cancer, an effective amount of an agent is, for example, an amount sufficient to achieve treatment, as defined herein, of cancer, as compared to the response obtained without administration of the agent.

[00782] The term "enantiomer," as used herein, means each individual optically active form of a compound of the invention, having an optical purity or enantiomeric excess (as determined by methods standard in the art) of at least 80% (i.e., at least 90% of one enantiomer and at most 10% of the other enantiomer), preferably at least 90% and more preferably at least 98%.

[00783] The term "halo," as used herein, represents a halogen selected from bromine, chlorine, iodine, or fluorine.

[00784] The term "haloalkoxy," as used herein, represents an alkoxy group, as defined herein, substituted by a halogen group (i.e., F, CI, Br, or I). A haloalkoxy may be substituted with one, two, three, or, in the case of alkyl groups of two carbons or more, four halogens. Haloalkoxy groups include perfluoroalkoxys (e.g., -OCF_3), -$OCHF_2$, -OCH_2F, -$OCCl_3$, -OCH_2CH_2Br, -$OCH_2CH(CH_2CH_2Br)CH_3$, and -$OCHICH_3$. In some embodiments, the haloalkoxy group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkyl groups.

[00785] The term "haloalkyl," as used herein, represents an alkyl group, as defined herein, substituted by a halogen group (i.e., F, CI, Br, or I). A haloalkyl may be substituted with one, two, three, or, in the case of alkyl groups of two carbons or more, four halogens. Haloalkyl groups include perfluoroalkyls (e.g., $-CF_3$), $-CHF_2$, $-CH_2F$, $-CCl_3$, $-CH_2CH_2Br$, $-CH_2CH(CH_2CH_2Br)CH_3$, and $-CHICH_3$. In some embodiments, the haloalkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkyl groups.

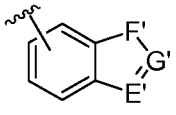
[00786] The term "heteroalkylene," as used herein, refers to an alkylene group, as defined herein, in which one or two of the constituent carbon atoms have each been replaced by nitrogen, oxygen, or sulfur. In some embodiments, the heteroalkylene group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkylene groups.

[00787] The term "heteroaryl," as used herein, represents that subset of heterocyclyls, as defined herein, which are aromatic: i.e., they contain *4n+2* pi electrons within the mono- or multicyclic ring system. Exemplary unsubstituted heteroaryl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. In some embodiment, the heteroaryl is substituted with 1, 2, 3, or 4 substituents groups as defined for a heterocyclyl group.

[00788] The term "heterocyclyl," as used herein represents a 5-, 6- or 7-membered ring, unless otherwise specified, containing one, two, three, or four heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. The 5-membered ring has zero to two double bonds, and the 6- and 7-membered rings have zero to three double bonds. Exemplary unsubstituted heterocyclyl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term "heterocyclyl" also represents a heterocyclic compound having a bridged multicyclic structure in which one or more carbons and/or heteroatoms bridges two non-adjacent members of a monocyclic ring, e.g., a quinuclidinyl group. The term "heterocyclyl" includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heterocyclic rings is fused to one, two, or three carbocyclic rings, e.g., an aryl ring, a cyclohexane ring, a cyclohexene ring, a cyclopentane ring, a cyclopentene ring, or another monocyclic heterocyclic ring, such as indolyl, quinolyl, isoquinolyl, tetrahydroquinolyl, benzofuryl, benzothienyl and the like.

Examples of fused heterocyclyls include tropanes and l,2,3,5,8,8a-hexahydroindolizine. Heterocyclics include pyrrolyl, pyrrolinyl, pyrrolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl, piperidinyl, homopiperidinyl, pyrazinyl, piperazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidiniyl, morpholinyl, thiomorpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, indolyl, indazolyl, quinolyl, isoquinolyl, quinoxalinyl, dihydroquinoxalinyl, quinazolinyl, cinnolinyl, phthalazinyl, benzimidazolyl, benzothiazolyl, benzoxazolyl, benzothiadiazolyl, furyl, thienyl, thiazolidinyl, isothiazolyl, triazolyl, tetrazolyl, oxadiazolyl (e.g., 1,2,3-oxadiazolyl), purinyl, thiadiazolyl (e.g., 1,2,3-thiadiazolyl), tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, dihydrothienyl, dihydroindolyl, dihydroquinolyl, tetrahydroquinolyl, tetrahydroisoquinolyl, dihydroisoquinolyl, pyranyl, dihydropyranyl, dithiazolyl, benzofuranyl, isobenzofuranyl, benzothienyl, and the like, including dihydro and tetrahydro forms thereof, where one or more double bonds are reduced and replaced with hydrogens. Still other exemplary heterocyclyls include: 2,3,4,5-tetrahydro-2-oxo-oxazolyl; 2,3-dihydro-2-oxo-lH-imidazolyl; 2,3,4,5-tetrahydro-5-oxo-lH-pyrazolyl (e.g., 2,3,4,5-tetrahydro-2-phenyl-5-oxo-lH-pyrazolyl); 2,3,4,5-tetrahydro-2,4-dioxo-lH-imidazolyl (e.g., 2,3,4,5-tetrahydro-2,4-dioxo-5-methyl-5-phenyl-lH-imidazolyl); 2,3-dihydro-2-thioxo-l,3,4-oxadiazolyl (e.g., 2,3-dihydro-2-thioxo-5-phenyl-l,3,4-oxadiazolyl); 4,5-dihydro-5-oxo-l *H*-triazolyl (e.g., 4,5-dihydro-3-methyl-4-amino 5-oxo-l*H*-triazolyl); l,2,3,4-tetrahydro-2,4-dioxopyridinyl (e.g., l,2,3,4-tetrahydro-2,4-dioxo-3,3-diethylpyridinyl); 2,6-dioxo-piperidinyl (e.g., 2,6-dioxo-3-ethyl-3-phenylpiperidinyl); l,6-dihydro-6-oxopyridiminyl; l,6-dihydro-4-oxopyrimidinyl (e.g., 2-(methylthio)-l,6-dihydro-4-oxo-5-methylpyrimidin-l-yl); l,2,3,4-tetrahydro-2,4-dioxopyrimidinyl (e.g., 1,2,3,4-tetrahydro-2,4-dioxo-3-ethylpyrimidinyl); 1,6-dihydro-6-oxo-pyridazinyl (e.g., l,6-dihydro-6-oxo-3-ethylpyridazinyl); l,6-dihydro-6-oxo-l,2,4-triazinyl (e.g., 1,6-dihydro-5-isopropyl-6-oxo-l,2,4-triazinyl); 2,3-dihydro-2-oxo-l *H*-indolyl (e.g., 3,3-dimethyl-2,3-dihydro-2-oxo-l *H*-indolyl and 2,3-dihydro-2-oxo-3,3'-spiropropane-l *H*-indol-1-yl); 1,3-dihydro-1-oxo-2*H*-iso-indolyl; 1,3-dihydro-1,3-dioxo-2*H*-iso-indolyl; l*H*-benzopyrazolyl (e.g., l-(ethoxycarbonyl)-l*H*-benzopyrazolyl); 2,3-dihydro-2-oxo-l*H*-benzimidazolyl (e.g., 3-ethyl-2,3-dihydro-2-oxo-l *H*-benzimidazolyl); 2,3-dihydro-2-

oxo-benzoxazolyl (e.g., 5-chloro-2,3-dihydro-2-oxo-benzoxazolyl); 2,3-dihydro-2-oxo-benzoxazolyl; 2-oxo-2H-benzopyranyl; 1,4-benzodioxanyl; 1,3-benzodioxanyl; 2,3-dihydro-3-oxo,4*H*-l,3-benzothiazinyl; 3,4-dihydro-4-oxo-3*H*-quinazolinyl (e.g., 2-methyl-3,4-dihydro-4-oxo-3*H*-quinazolinyl); 1,2,3,4-tetrahydro-2,4-dioxo-3*H*-quinazolyl (e.g., 1-ethyl-1,2,3,4-tetrahydro-2,4-dioxo-3*H*-quinazolyl); l,2,3,6-tetrahydro-2,6-dioxo-7*H*-purinyl (e.g., 1,2,3,6-tetrahydro-l,3-dimethyl-2, 6-dioxo-7*H*-purinyl); 1,2,3,6-tetrahydro-2,6-dioxo-l*H*-purinyl (e.g., l,2,3,6-tetrahydro-3,7-dimethyl-2,6-dioxo-l*H*-purinyl); 2-oxobenz[c,d]indolyl; l,l-dioxo-2H-naphth[l,8-c,d]isothiazolyl; and 1,8-naphthylenedicarboxamido. Additional heterocyclics include 3,3a,4,5,6,6a-hexahydro-pyrrolo[3,4-b]pyrrol-(2H)-yl, and 2,5-diazabicyclo[2.2.1]heptan-2-yl, homopiperazinyl (or diazepanyl), tetrahydropyranyl, dithiazolyl, benzofuranyl, benzothienyl, oxepanyl, thiepanyl, azocanyl, oxecanyl, and thiocanyl. Heterocyclic groups also include groups of the formula

[00789] 

, where

[00790] E' is selected from the group consisting of -N- and **-CH-;** F' is selected from the group consisting of **-N=CH-, -NH-CH$_2$-, -NH-C(O)-, -NH-, -CH=N-, -CH$_2$-NH-, -C(0)-NH-, -CH=CH-, -CH$_2$-, -CH$_2$CH$_2$-, -CH$_2$0** -, **-OCH$_2$-,** -0-, and **-S-;** and **G'** is selected from the group consisting of **-CH-** and **-N-.** Any of the heterocyclyl groups mentioned herein may be optionally substituted with one, two, three, four or five substituents independently selected from the group consisting of: (1) **Ci$_{-7}$** acyl (e.g., carboxyaldehyde); (2) **Ci$_{-2}$o** alkyl (e.g., **Ci$_{-6}$** alkyl, **Ci$_{-6}$ alkoxy-Ci$_{-6}$** alkyl, **Ci$_{-6}$** alkylsulfmyl-**Ci$_{-6}$** alkyl, amino-**Ci$_{-6}$** alkyl, **azido-Ci$_{-6}$** alkyl, (carboxyaldehyde)-**Ci$_{-6}$** alkyl, halo-**Ci$_{-6}$** alkyl (e.g., perfluoroalkyl), hydroxy-**Ci$_{-6}$** alkyl, nitro-**Ci$_{-6}$** alkyl, or **Ci$_{-6}$** thioalkoxy-**Ci$_{-6}$** alkyl); (3) **Ci$_{-20}$** alkoxy (e.g., **Ci$_{-6}$** alkoxy, such as perfluoroalkoxy); (4) **Ci$_{-6}$** alkylsulfinyl; (5) **C$_{6-1}$o** aryl; (6) amino; (7) **Ci$_{-6}$ alk-C$_{6-io}$** aryl; (8) azido; (9) C3-8 cycloalkyl; (10) **Ci$_{-6}$** alk-**C**$_{3-8}$ cycloalkyl; (11) halo; (12) **C$_{1-12}$** heterocyclyl (e.g., **C$_{2-i2}$** heteroaryl); (13) **(C$_{1-12}$** heterocyclyl)oxy; (14) hydroxy; (15) nitro; (16) **Ci$_{-2}$o** thioalkoxy (e.g., **Ci$_{-6}$** thioalkoxy); (17) **-(CH$_2$)$_q$C0 $_2$R$^{A'}$**, where q is an integer from zero to four, and **R$^{A'}$** is selected from the group consisting of (a) **Ci$_{-6}$** alkyl, (b) **C$_{6-1}$o** aryl, (c) hydrogen, and (d) **Ci$_{-6}$ alk-C$_{6-io}$** aryl; (18) **-(CH$_2$)$_q$CONR$^{B'}$R$^{C'}$**, where q is an integer from zero to

four and where $R^{B'}$ and $R^{C'}$ are independently selected from the group consisting of (a) hydrogen, (b) Ci_6 alkyl, (c) C_{6-i0} aryl, and (d) Ci_6 alk-C_{6-i0} aryl; (19) -$(CH_2)_qS0_2R^{D'}$, where q is an integer from zero to four and where $R^{D'}$ is selected from the group consisting of (a) Ci_6 alkyl, (b) C_6-io aryl, and (c) Ci_6 alk-C_6-io aryl; (20) -$(CH_2)_qS0_2NR^{E'}R^{F'}$, where q is an integer from zero to four and where each of $R^{E'}$ and $R^{F'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) Ci_6 alkyl, (c) C_6-io aryl, and (d) Ci_6 alk-C_6-io aryl; (21) thiol; (22) C_6-io aryloxy; (23) C3-8 cycloalkoxy; (24) arylalkoxy; (25) Ci_6 alk-Ci_i2 heterocyclyl (e.g., Ci_6 alk-Ci_i2 heteroaryl); (26) oxo; (27) (Ci_i2 heterocyclyl)imino; (28) C_{2-2}o alkenyl; and (29) C_{2-2}o alkynyl. In some embodiments, each of these groups can be further substituted as described herein. For example, the alkylene group of a Ci-alkaryl or a Ci-alkheterocyclyl can be further substituted with an oxo group to afford the respective aryloyl and (heterocyclyl)oyl substituent group.

[00791] The term "(heterocyclyl)imino," as used herein, represents a heterocyclyl group, as defined herein, attached to the parent molecular group through an imino group. In some embodiments, the heterocyclyl group can be substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00792] The term "(heterocyclyl)oxy," as used herein, represents a heterocyclyl group, as defined herein, attached to the parent molecular group through an oxygen atom. In some embodiments, the heterocyclyl group can be substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00793] The term "(heterocyclyl)oyl," as used herein, represents a heterocyclyl group, as defined herein, attached to the parent molecular group through a carbonyl group. In some embodiments, the heterocyclyl group can be substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00794] The term "hydrocarbon," as used herein, represents a group consisting only of carbon and hydrogen atoms.

[00795] The term "hydroxy," as used herein, represents an -OH group.

[00796] The term "hydroxyalkenyl," as used herein, represents an alkenyl group, as defined herein, substituted by one to three hydroxy groups, with the proviso that no more

than one hydroxy group may be attached to a single carbon atom of the alkyl group, and is exemplified by dihydroxypropenyl, hydroxyisopentenyl, and the like.

[00797] The term "hydroxyalkyl," as used herein, represents an alkyl group, as defined herein, substituted by one to three hydroxy groups, with the proviso that no more than one hydroxy group may be attached to a single carbon atom of the alkyl group, and is exemplified by hydroxymethyl, dihydroxypropyl, and the like.

[00798] The term "isomer," as used herein, means any tautomer, stereoisomer, enantiomer, or diastereomer of any compound of the invention. It is recognized that the compounds of the invention can have one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric E/Z isomers) or diastereomers (e.g., enantiomers (i.e., (+) or (-)) or cis/trans isomers). According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all of the corresponding stereoisomers, that is, both the stereomerically pure form (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures, e.g., racemates. Enantiomeric and stereoisomeric mixtures of compounds of the invention can typically be resolved into their component enantiomers or stereoisomers by well-known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically or enantiomerically pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

[00799] The term "*N*-protected amino," as used herein, refers to an amino group, as defined herein, to which is attached one or two *N*-protecting groups, as defined herein.

[00800] The term "*N*-protecting group," as used herein, represents those groups intended to protect an amino group against undesirable reactions during synthetic procedures. Commonly used *N*-protecting groups are disclosed in Greene, "Protective Groups in Organic Synthesis," 3rd Edition (John Wiley & Sons, New York, 1999), which is incorporated herein by reference. *N*-protecting groups include acyl, aryloyl, or carbamyl groups such as formyl, acetyl, propionyl, pivaloyl, t-butylacetyl, 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, phthalyl, o-nitrophenoxyacetyl, a-

chlorobutyryl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl, and chiral auxiliaries such as protected or unprotected D, L or D, L-amino acids such as alanine, leucine, phenylalanine, and the like; sulfonyl-containing groups such as benzenesulfonyl, p-toluenesulfonyl, and the like; carbamate forming groups such as benzyloxycarbonyl, p-chlorobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 3,5-dimethoxybenzyloxycarbonyl, 2,4-dimethoxybenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, l-(p-biphenylyl)-l-methylethoxycarbonyl, α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxy carbonyl, t-butyloxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl, 2,2,2,-trichloroethoxycarbonyl, phenoxycarbonyl, 4-nitrophenoxy carbonyl, fluorenyl-9-methoxycarbonyl, cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl, phenylthiocarbonyl, and the like, alkaryl groups such as benzyl, triphenylmethyl, benzyloxymethyl, and the like and silyl groups, such as trimethylsilyl, and the like. Preferred *N*-protecting groups are formyl, acetyl, benzoyl, pivaloyl, t-butylacetyl, alanyl, phenylsulfonyl, benzyl, t-butyloxycarbonyl (Boc), and benzyloxycarbonyl (Cbz).

[00801] The term "nitro," as used herein, represents an -N0 $_2$ group.

[00802] The term "oxo" as used herein, represents =0.

[00803] The term "perfluoroalkyl," as used herein, represents an alkyl group, as defined herein, where each hydrogen radical bound to the alkyl group has been replaced by a fluoride radical. Perfluoroalkyl groups are exemplified by trifluoromethyl, pentafluoroethyl, and the like.

[00804] The term "perfluoroalkoxy," as used herein, represents an alkoxy group, as defined herein, where each hydrogen radical bound to the alkoxy group has been replaced by a fluoride radical. Perfluoroalkoxy groups are exemplified by trifluoromethoxy, pentafluoroethoxy, and the like.

[00805] The term "spirocyclyl," as used herein, represents a C_{2-7} alkylene diradical, both ends of which are bonded to the same carbon atom of the parent group to form a spirocyclic group, and also a **Ci**$_{-6}$ heteroalkylene diradical, both ends of which are bonded

to the same atom. The heteroalkylene radical forming the spirocyclyl group can containing one, two, three, or four heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, the spirocyclyl group includes one to seven carbons, excluding the carbon atom to which the diradical is attached. The spirocyclyl groups of the invention may be optionally substituted with 1, 2, 3, or 4 substituents provided herein as optional substituents for cycloalkyl and/or heterocyclyl groups.

[00806] The term "stereoisomer," as used herein, refers to all possible different isomeric as well as conformational forms which a compound may possess (e.g., a compound of any formula described herein), in particular all possible stereochemically and conformationally isomeric forms, all diastereomers, enantiomers and/or conformers of the basic molecular structure. Some compounds of the present invention may exist in different tautomeric forms, all of the latter being included within the scope of the present invention.

[00807] The term "sulfoalkyl," as used herein, represents an alkyl group, as defined herein, substituted by a sulfo group of $-SO_3H$. In some embodiments, the alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00808] The term "sulfonyl," as used herein, represents an $-S(0)_2-$ group.

[00809] The term "thioalkaryl," as used herein, represents a chemical substituent of formula -SR, where R is an alkaryl group. In some embodiments, the alkaryl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00810] The term "thioalkheterocyclyl," as used herein, represents a chemical substituent of formula -SR, where R is an alkheterocyclyl group. In some embodiments, the alkheterocyclyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00811] The term "thioalkoxy," as used herein, represents a chemical substituent of formula -SR, where R is an alkyl group, as defined herein. In some embodiments, the alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00812] The term "thiol" represents an -SH group.

[00813] *Compound:* As used herein, the term "compound," is meant to include all stereoisomers, geometric isomers, tautomers, and isotopes of the structures depicted.

[00814] The compounds described herein can be asymmetric *{e.g.*, having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless otherwise indicated. Compounds of the present disclosure that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present disclosure. Cis and trans geometric isomers of the compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms.

[00815] Compounds of the present disclosure also include tautomeric forms. Tautomeric forms result from the swapping of a single bond with an adjacent double bond and the concomitant migration of a proton. Tautomeric forms include prototropic tautomers which are isomeric protonation states having the same empirical formula and total charge. Examples prototropic tautomers include ketone - enol pairs, amide - imidic acid pairs, lactam - lactim pairs, amide - imidic acid pairs, enamine - imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, such as, IH- and 3H-imidazole, 1H-, 2H- and 4H- 1,2,4-triazole, IH- and 2H- isoindole, and IH- and 2H-pyrazole. Tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution.

[00816] Compounds of the present disclosure also include all of the isotopes of the atoms occurring in the intermediate or final compounds. "Isotopes" refers to atoms having the same atomic number but different mass numbers resulting from a different number of neutrons in the nuclei. For example, isotopes of hydrogen include tritium and deuterium.

[00817] The compounds and salts of the present disclosure can be prepared in combination with solvent or water molecules to form solvates and hydrates by routine methods.

[00818] *Condition:* As used herein, the term "condition" refers to a disorder that presents with observable symptoms.

[00819] *Conserved:* As used herein, the term "conserved" refers to nucleotides or amino acid residues of a polynucleotide sequence or polypeptide sequence, respectively, that are those that occur unaltered in the same position of two or more sequences being compared. Nucleotides or amino acids that are relatively conserved are those that are conserved amongst more related sequences than nucleotides or amino acids appearing elsewhere in the sequences.

[00820] In some embodiments, two or more sequences are said to be "completely conserved" if they are 100% identical to one another. In some embodiments, two or more sequences are said to be "highly conserved" if they are at least 70%> identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some embodiments, two or more sequences are said to be "highly conserved" if they are about **70%o** identical, about 80%> identical, about 90%> identical, about 95%, about 98%>, or about 99% identical to one another. In some embodiments, two or more sequences are said to be "conserved" if they are at least 30% identical, at least 40% identical, at least 50% identical, at least 60% identical, at least 70% identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some embodiments, two or more sequences are said to be "conserved" if they are about 30%> identical, about 40%> identical, about 50%> identical, about 60%> identical, about 70%> identical, about 80%> identical, about 90%> identical, about 95% identical, about 98%> identical, or about 99% identical to one another. Conservation of sequence may apply to the entire length of an oligonucleotide or polypeptide or may apply to a portion, region or feature thereof.

[00821] *Cyclic or Cyclized:* As used herein, the term "cyclic" refers to the presence of a continuous loop. Cyclic molecules need not be circular, only joined to form an unbroken chain of subunits. Cyclic molecules such as the engineered RNA or mRNA of the present invention may be single units or multimers or comprise one or more components of a complex or higher order structure.

[00822] *Cytostatic:* As used herein, "cytostatic" refers to inhibiting, reducing, suppressing the growth, division, or multiplication of a cell *{e.g.,* a mammalian cell *{e.g.,*

a human cell)), bacterium, virus, fungus, protozoan, parasite, prion, or a combination thereof.

[00823] *Cytotoxic:* As used herein, "cytotoxic" refers to killing or causing injurious, toxic, or deadly effect on a cell *{e.g.,* a mammalian cell *{e.g.,* a human cell)), bacterium, virus, fungus, protozoan, parasite, prion, or a combination thereof.

[00824] *Delivery:* As used herein, "delivery" refers to the act or manner of delivering a compound, substance, entity, moiety, cargo or payload.

[00825] *Delivery Agent:* As used herein, "delivery agent" refers to any substance which facilitates, at least in part, the *in vivo* delivery of signal-sensor polynucleotide, primary construct or mmRNA to targeted cells.

[00826] *Destabilized:* As used herein, the term "destable," "destabilize," or "destabilizing region" means a region or molecule that is less stable than a starting, wild-type or native form of the same region or molecule.

[00827] *Detectable label:* As used herein, "detectable label" refers to one or more markers, signals, or moieties which are attached, incorporated or associated with another entity that is readily detected by methods known in the art including radiography, fluorescence, chemiluminescence, enzymatic activity, absorbance and the like. Detectable labels include radioisotopes, fluorophores, chromophores, enzymes, dyes, metal ions, ligands such as biotin, avidin, streptavidin and haptens, quantum dots, and the like. Detectable labels may be located at any position in the peptides or proteins disclosed herein. They may be within the amino acids, the peptides, or proteins, or located at the N- or C- termini.

[00828] *Disease:* As used herein, the term "disease" refers to an abnormal condition affecting the body of an organism often showing specific bodily symptoms.

[00829] *Disorder:* As used herein, the term "disorder, "refers to a disruption of or an interference with normal functions or established systems of the body.

[00830] *Digest:* As used herein, the term "digest" means to break apart into smaller pieces or components. When referring to polypeptides or proteins, digestion results in the production of peptides.

[00831] *Distal:* As used herein, the term "distal" means situated away from the center or away from a point or region of interest.

[00832] *Dose splitting factor (DSF)*-ratio of PUD of dose split treatment divided by PUD of total daily dose or single unit dose. The value is derived from comparison of dosing regimens groups.

[00833] *Encoded protein cleavage signal:* As used herein, "encoded protein cleavage signal" refers to the nucleotide sequence which encodes a protein cleavage signal.

[00834] *Engineered:* As used herein, embodiments of the invention are "engineered" when they are designed to have a feature or property, whether structural or chemical, that varies from a starting point, wild type or native molecule.

[00835] *Exosome:* As used herein, "exosome" is a vesicle secreted by mammalian cells or a complex involved in RNA degradation.

[00836] *Expression:* As used herein, "expression" of a nucleic acid sequence refers to one or more of the following events: (1) production of an RNA template from a DNA sequence *(e.g.,* by transcription); (2) processing of an RNA transcript *(e.g.,* by splicing, editing, 5' cap formation, and/or 3' end processing); (3) translation of an RNA into a polypeptide or protein; and (4) post-translational modification of a polypeptide or protein.

[00837] *Feature:* As used herein, a "feature" refers to a characteristic, a property, or a distinctive element.

[00838] *Formulation:* As used herein, a "formulation" includes at least a signal-sensor polynucleotide, primary construct or mmRNA and a delivery agent.

[00839] *Fragment:* A "fragment," as used herein, refers to a portion. For example, fragments of proteins may comprise polypeptides obtained by digesting full-length protein isolated from cultured cells.

[00840] *Functional:* As used herein, a "functional" biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized.

[00841] *Genotype: As used herein, "genotype" refers to the change in the genotype, or genetic makeup, of a subject, cell, tissue, organ and/or organism.*

[00842] *Homology:* As used herein, the term "homology" refers to the overall relatedness between polymeric molecules, *e.g.* between nucleic acid molecules *(e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be "homologous" to one another if

their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical or similar. The term "homologous" necessarily refers to a comparison between at least two sequences (polynucleotide or polypeptide sequences). In accordance with the invention, two polynucleotide sequences are considered to be homologous if the polypeptides they encode are at least about 50%>, 60%, 70%, 80%, 90%, 95%, or even 99% for at least one stretch of at least about 20 amino acids. In some embodiments, homologous polynucleotide sequences are characterized by the ability to encode a stretch of at least 4-5 uniquely specified amino acids. For polynucleotide sequences less than 60 nucleotides in length, homology is determined by the ability to encode a stretch of at least 4-5 uniquely specified amino acids. In accordance with the invention, two protein sequences are considered to be homologous if the proteins are at least about 50%>, 60%>, 70%>, 80%>, or 90%> identical for at least one stretch of at least about 20 amino acids.

[00843] *Identity:* As used herein, the term "identity" refers to the overall relatedness between polymeric molecules, *e.g.,* between oligonucleotide molecules *{e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of the percent identity of two polynucleotide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes *{e.g.,* gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%>, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide

sequences can be determined using methods such as those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; each of which is incorporated herein by reference. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4:1 1-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix. Methods commonly employed to determine percent identity between sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., SIAM J Applied Math., 48:1073 (1988); incorporated herein by reference. Techniques for determining identity are codified in publicly available computer programs. Exemplary computer software to determine homology between two sequences include, but are not limited to, GCG program package, Devereux, J., *et al., Nucleic Acids Research,* 12(1), 387 (1984)), BLASTP, BLASTN, and FASTA Altschul, S. F. *et al., J. Molec. Biol,* 215, 403 (1990)).

[00844] *Inhibit expression of a gene:* As used herein, the phrase "inhibit expression of a gene" means to cause a reduction in the amount of an expression product of the gene. The expression product can be an RNA transcribed from the gene *{e.g.,* an mRNA) or a polypeptide translated from an mRNA transcribed from the gene. Typically a reduction in the level of an mRNA results in a reduction in the level of a polypeptide translated therefrom. The level of expression may be determined using standard techniques for measuring mRNA or protein.

[00845] *In vitro:* As used herein, the term *"in vitro"* refers to events that occur in an artificial environment, *e.g.,* in a test tube or reaction vessel, in cell culture, in a Petri dish, *etc.,* rather than within an organism *{e.g.,* animal, plant, or microbe).

[00846] *In vivo:* As used herein, the term *"in vivo"* refers to events that occur within an organism *{e.g.,* animal, plant, or microbe or cell or tissue thereof).

[00847] *Isolated:* As used herein, the term "isolated" refers to a substance or entity that has been separated from at least some of the components with which it was associated (whether in nature or in an experimental setting). Isolated substances may have varying levels of purity in reference to the substances from which they have been associated. Isolated substances and/or entities may be separated from at least about 10%, about 20%>, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In some embodiments, isolated agents are more than about 80%>, about 85%, about 90%>, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. *Substantially isolated:* By "substantially isolated" is meant that the compound is substantially separated from the environment in which it was formed or detected. Partial separation can include, for example, a composition enriched in the compound of the present disclosure. Substantial separation can include compositions containing at least about 50%, at least about 60%, at least about 70%, at least about 80%>, at least about 90%>, at least about 95%, at least about 97%, or at least about 99%, by weight of the compound of the present disclosure, or salt thereof. Methods for isolating compounds and their salts are routine in the art.

[00848] *Linker:* As used herein, a linker refers to a group of atoms, e.g., 10-1,000 atoms, and can be comprised of the atoms or groups such as, but not limited to, carbon, amino, alkylamino, oxygen, sulfur, sulfoxide, sulfonyl, carbonyl, and imine. The linker can be attached to a modified nucleoside or nucleotide on the nucleobase or sugar moiety at a first end, and to a payload, e.g., a detectable or therapeutic agent, at a second end. The linker may be of sufficient length as to not interfere with incorporation into a nucleic acid sequence. The linker can be used for any useful purpose, such as to form mmRNA multimers (e.g., through linkage of two or more signal-sensor polynucleotides, primary constructs, or mmRNA molecules) or mmRNA conjugates, as well as to administer a payload, as described herein. Examples of chemical groups that can be incorporated into the linker include, but are not limited to, alkyl, alkenyl, alkynyl, amido, amino, ether,

thioether, ester, alkylene, heteroalkylene, aryl, or heterocyclyl, each of which can be optionally substituted, as described herein. Examples of linkers include, but are not limited to, unsaturated alkanes, polyethylene glycols (e.g., ethylene or propylene glycol monomeric units, e.g., diethylene glycol, dipropylene glycol, triethylene glycol, tripropylene glycol, tetraethylene glycol, or tetraethylene glycol), and dextran polymers, Other examples include, but are not limited to, cleavable moieties within the linker, such as, for example, a disulfide bond (-S-S-) or an azo bond (-N=N-), which can be cleaved using a reducing agent or photolysis. Non-limiting examples of a selectively cleavable bond include an amido bond can be cleaved for example by the use of tris(2-carboxyethyl)phosphine (TCEP), or other reducing agents, and/or photolysis, as well as an ester bond can be cleaved for example by acidic or basic hydrolysis.

[00849] *Metastasis:* As used herein, the term "metastasis" means the process by which cancer spreads from the place at which it first arose as a primary tumor to distant locations in the body.

[00850] *Method of Treating:* The phrase "a method of treating" or its equivalent, when applied to, for example, cancer refers to a procedure or course of action that is designed to reduce or eliminate the number of cancer cells, prevent the increase in the number of cancer cells, or to alleviate the symptoms of a cancer in a subject. A method of treating cancer or another oncology-related disorder does not necessarily mean that the cancer cells or other disorder will, in fact, be completely eliminated, that the number of cells or disorder will, in fact, be reduced, or that the symptoms of a cancer or other disorder will, in fact, be alleviated. Often, a method of treating cancer will be performed even with a low likelihood of success, but which, given the medical history and estimated survival expectancy of a subject, is nevertheless deemed an overall beneficial course of action.

[00851] *MicroRNA (miRNA) binding site:* As used herein, a microRNA (miRNA) binding site represents a nucleotide location or region of a nucleic acid transcript to which at least the "seed" region of a miRNA binds.

[00852] *Modified:* As used herein "modified" refers to a changed state or structure of a molecule of the invention. Molecules may be modified in many ways including chemically, structurally, and functionally. In one embodiment, the mRNA molecules of the present invention are modified by the introduction of non-natural nucleosides and/or

nucleotides, e.g., as it relates to the natural ribonucleotides A, U, G, and C. Noncanonical nucleotides such as the cap structures are not considered "modified" although they differ from the chemical structure of the A, C, G, U ribonucleotides.

[00853] *Mucus:* As used herein, "mucus" refers to the natural substance that is viscous and comprises mucin glycoproteins.

[00854] *Naturally occurring:* As used herein, "naturally occurring" means existing in nature without artificial aid.

[00855] *Non-human vertebrate:* As used herein, a "non human vertebrate" includes all vertebrates except *Homo sapiens,* including wild and domesticated species. Examples of non-human vertebrates include, but are not limited to, mammals, such as alpaca, banteng, bison, camel, cat, cattle, deer, dog, donkey, gayal, goat, guinea pig, horse, llama, mule, pig, rabbit, reindeer, sheep water buffalo, and yak.

[00856] *Off-target:* As used herein, "off target" refers to any unintended effect on any one or more target, gene, or cellular transcript.

[00857] *Oncology-related:* As used herein, the term "oncology-related" refers to any disease, disorder, condition, treatment, process, substance or compound related to any aspect of one or more hyperproliferative diseases, disorders and/or conditions including, but not limited to, cancer.

[00858] *Open reading frame:* As used herein, "open reading frame" or "ORF" refers to a sequence which does not contain a stop codon in a given reading frame.

[00859] *Operably linked:* As used herein, the phrase "operably linked" refers to a functional connection between two or more molecules, constructs, transcripts, entities, moieties or the like.

[00860] *Paratope:* As used herein, a "paratope" refers to the antigen-binding site of an antibody.

[00861] *Patient:* As used herein, "patient" refers to a subject who may seek or be in need of treatment, requires treatment, is receiving treatment, will receive treatment, or a subject who is under care by a trained professional for a particular disease or condition.

[00862] *Optionally substituted:* Herein a phrase of the form "optionally substituted X" *{e.g.,* optionally substituted alkyl) is intended to be equivalent to "X, wherein X is

optionally substituted" *(e.g.*, "alkyl, wherein said alkyl is optionally substituted"). It is not intended to mean that the feature "X" *(e.g.* alkyl) *per se* is optional.

[00863] *Peptide:* As used herein, "peptide" is less than or equal to 50 amino acids long, e.g., about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

[00864] *Pharmaceutical composition:* The phrase "pharmaceutical composition" refers to a composition that alters the etiology of a disease, disorder and/or condition.

[00865] *Pharmaceutically acceptable:* The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00866] *Pharmaceutically acceptable excipients:* The phrase "pharmaceutically acceptable excipient," as used herein, refers any ingredient other than the compounds described herein (for example, a vehicle capable of suspending or dissolving the active compound) and having the properties of being substantially nontoxic and non-inflammatory in a patient. Excipients may include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, and waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

[00867] *Pharmaceutically acceptable salts:* The present disclosure also includes pharmaceutically acceptable salts of the compounds described herein. As used herein, "pharmaceutically acceptable salts" refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form (e.g., by reacting the free base group with a suitable organic acid). Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. The pharmaceutically acceptable salts of the present disclosure include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present disclosure can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences,* 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418, *Pharmaceutical Salts:*

Properties, Selection, and Use, P.H. Stahl and C.G. Wermuth (eds.), Wiley-VCH, 2008, and Berge et al., *Journal of Pharmaceutical Science,* 66, 1-19 (1977), each of which is incorporated herein by reference in its entirety.

[00868] *Pharmaceutically acceptable solvate:* The term "pharmaceutically acceptable solvate," as used herein, means a compound of the invention wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered. For example, solvates may be prepared by crystallization, recrystallization, or precipitation from a solution that includes organic solvents, water, or a mixture thereof. Examples of suitable solvents are ethanol, water (for example, mono-, di-, and tri-hydrates), *N*-methylpyrrolidinone (NMP), dimethyl sulfoxide (DMSO), *N,N* '-dimethylformamide (DMF), *N,N* '-dimethylacetamide (DMAC), l,3-dimethyl-2-imidazolidinone (DMEU), l,3-dimethyl-3,4,5,6-tetrahydro-2-(lH)-pyrimidinone (DMPU), acetonitrile (ACN), propylene glycol, ethyl acetate, benzyl alcohol, 2-pyrrolidone, benzyl benzoate, and the like. When water is the solvent, the solvate is referred to as a "hydrate."

[00869] *Pharmacokinetic:* As used herein, "pharmacokinetic" refers to any one or more properties of a molecule or compound as it relates to the determination of the fate of substances administered to a living organism. Pharmacokinetics is divided into several areas including the extent and rate of absorption, distribution, metabolism and excretion. This is commonly referred to as ADME where: (A) Absorption is the process of a substance entering the blood circulation; (D) Distribution is the dispersion or dissemination of substances throughout the fluids and tissues of the body; (M) Metabolism (or Biotransformation) is the irreversible transformation of parent compounds into daughter metabolites; and (E) Excretion (or Elimination) refers to the elimination of the substances from the body. In rare cases, some drugs irreversibly accumulate in body tissue.

[00870] *Phenotype:* As used herein, "phenotype" refers to the set of observable characteristics of a subject, cell, tissue, organ and/or organism.

[00871] *Physicochemical:* As used herein, "physicochemical" means of or relating to a physical and/or chemical property.

[00872] *Preventing:* As used herein, the term "preventing" refers to partially or completely delaying onset of an infection, disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying progression from an infection, a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the infection, the disease, disorder, and/or condition.

[00873] *Prodrug:* The present disclosure also includes prodrugs of the compounds described herein. As used herein, "prodrugs" refer to any substance, molecule or entity which is in a form predicate for that substance, molecule or entity to act as a therapeutic upon chemical or physical alteration. Prodrugs may by covalently bonded or sequestered in some way and which release or are converted into the active drug moiety prior to, upon or after administered to a mammalian subject. Prodrugs can be prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent compounds. Prodrugs include compounds wherein hydroxyl, amino, sulfhydryl, or carboxyl groups are bonded to any group that, when administered to a mammalian subject, cleaves to form a free hydroxyl, amino, sulfhydryl, or carboxyl group respectively. Preparation and use of prodrugs is discussed in T. Higuchi and V. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in *Bioreversible Carriers in Drug Design,* ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are hereby incorporated by reference in their entirety.

[00874] *Proliferate:* As used herein, the term "proliferate" means to grow, expand or increase or cause to grow, expand or increase rapidly. "Proliferative" means having the ability to proliferate. "Anti-proliferative" means having properties counter to or inapposite to proliferative properties.

[00875] *Protein cleavage site:* As used herein, "protein cleavage site" refers to a site where controlled cleavage of the amino acid chain can be accomplished by chemical, enzymatic or photochemical means.

[00876] *Protein cleavage signal:* As used herein "protein cleavage signal" refers to at least one amino acid that flags or marks a polypeptide for cleavage.

[00877] *Progression:* As used herein, the term "progression" (e.g., cancer progression) means the advancement or worsening of or toward a disease or condition.

[00878] *Protein of interest:* As used herein, the terms "proteins of interest" or "desired proteins" include those provided herein and fragments, mutants, variants, and alterations thereof.

[00879] *Proximal:* As used herein, the term "proximal" means situated nearer to the center or to a point or region of interest.

[00880] *Pseudouridine:* As used herein, pseudouridine refers to the C-glycoside isomer of the nucleoside uridine. A "pseudouridine analog" is any modification, variant, isoform or derivative of pseudouridine. For example, pseudouridine analogs include but are not limited to 1-carboxymethyl-pseudouridine, 1-propynyl-pseudouridine, 1-taurinomethyl-pseudouridine, 1-taurinomethyl-4-thio-pseudouridine, 1-methyl-pseudouridine (m^), l-methyl-4-thio-pseudouridine **(mV ẇ),** 4-thio-l-methyl-pseudouridine, 3-methyl-pseudouridine ($m^3\psi$), 2-thio-l -methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-l -methyl- 1-deaza-pseudouridine, dihydropseudouridine, 2-thio-dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, l-methyl-3-(3-amino-3-carboxypropyl)pseudouridine ($acp^3\psi$), and 2'-**0** -methyl-pseudouridine (ψm).

[00881] *Purified:* As used herein, "purify," "purified," "purification" means to make substantially pure or clear from unwanted components, material defilement, admixture or imperfection.

[00882] *Regression:* As used herein, the term "regression" or "degree of regression" refers to the reversal, either phenotypically or genotypically, of a cancer progression. Slowing or stopping cancer progression may be considered regression.

[00883] *Reducing the effect:* As used herein, the phrase "reducing the effect" when referring to symptoms, means reducing, eliminating or alleviating the symptom in the

subject. It does not necessarily mean that the symptom will, in fact, be completely eliminated, reduced or alleviated.

[00884] *Sample:* As used herein, the term "sample" or "biological sample" refers to a subset of its tissues, cells or component parts (e.g. body fluids, including but not limited to blood, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen). A sample further may include a homogenate, lysate or extract prepared from a whole organism or a subset of its tissues, cells or component parts, or a fraction or portion thereof, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs. A sample further refers to a medium, such as a nutrient broth or gel, which may contain cellular components, such as proteins or nucleic acid molecule.

[00885] *Side effect:* As used herein, the phrase "side effect" refers to a secondary effect of treatment.

[00886] *Signal Peptide Sequences:* As used herein, the phrase "signal peptide sequences" refers to a sequence which can direct the transport or localization of a protein.

[00887] *Signal-sensor polynucleotide:* As used herein, "signal-sensor polynucleotides" are nucleic acid transcripts which encode one or more oncology-related polypeptides of interest that, when translated, delivers a "signal" to the cell (cancer or noncancerous) which results in the therapeutic benefit to the organism of either being detrimental to the cancer cell or beneficial to normal cells or both detrimental to cancer cells and advantageous to normal cells. The signal-sensor polynucleotides may optionally further comprise a sequence (translatable or not) which "senses" the microenvironment of the polynucleotide and alters (a) the function or phenotypic outcome associated with the peptide or protein which is translated, (b) the expression level of the signal-sensor polynucleotide, and/or both.

[00888] *Single unit dose:* As used herein, a "single unit dose" is a dose of any therapeutic administed in one dose/at one time/single route/single point of contact, i.e., single administration event.

[00889] *Similarity:* As used herein, the term "similarity" refers to the overall relatedness between polymeric molecules, *e.g.* between polynucleotide molecules *{e.g.*

DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of percent similarity of polymeric molecules to one another can be performed in the same manner as a calculation of percent identity, except that calculation of percent similarity takes into account conservative substitutions as is understood in the art.

[00890] *Skin:* The term "skin" is the thin layer of tissue forming the natural outer covering of the body of a subject and includes the epidermis and the dermis. The dermis is the thick layer of living tissue below the epidermis which is the surface epithelium of the skin.

[00891] *Split dose:* As used herein, a "split dose" is the division of single unit dose or total daily dose into two or more doses.

[00892] *Stable:* As used herein "stable" refers to a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and preferably capable of formulation into an efficacious therapeutic agent.

[00893] *Stabilized:* As used herein, the term "stabilize", "stabilized," "stabilized region" means to make or become stable.

[00894] *Subject:* As used herein, the term "subject" or "patient" refers to any organism to which a composition in accordance with the invention may be administered, *e.g.,* for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals *(e.g.,* mammals such as mice, rats, rabbits, non-human primates, and humans) and/or plants.

[00895] *Substantially:* As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[00896] *Substantially equal:* As used herein as it relates to time differences between doses, the term means plus/minus 2%.

[00897] *Substantially simultaneously:* As used herein and as it relates to plurality of doses, the term means within 2 seconds.

[00898] *Sufferingfrom:* An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with or displays one or more symptoms of a disease, disorder, and/or condition.

[00899] *Susceptible to:* An individual who is "susceptible to" a disease, disorder, and/or condition has not been diagnosed with and/or may not exhibit symptoms of the disease, disorder, and/or condition but harbors a propensity to develop a disease or its symptoms. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition (for example, cancer) may be characterized by one or more of the following: (1) a genetic mutation associated with development of the disease, disorder, and/or condition; (2) a genetic polymorphism associated with development of the disease, disorder, and/or condition; (3) increased and/or decreased expression and/or activity of a protein and/or nucleic acid associated with the disease, disorder, and/or condition; (4) habits and/or lifestyles associated with development of the disease, disorder, and/or condition; (5) a family history of the disease, disorder, and/or condition; and (6) exposure to and/or infection with a microbe associated with development of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[00900] *Symptom:* As used herein, the term "symptom" is a signal of a disease, disorder and/or condition. For example, symptoms may be felt or noticed by the subject who has them but may not be easily accessed by looking at a subject's outward appearance or behaviors. Examples of symptoms include, but are not limited to, weakness, aches and pains, fever, fatigue, weight loss, blood clots, increased blood calcium levels, low white blood cell count, short of breath, dizziness, headaches, hyperpigmentation, jaundice, erthema, pruritis, excessive hair growth, change in bowel habits, change in bladder function, long-lasting sores, white patches inside the mouth, white spots on the tongue, unusual bleeding or discharge, thickening or lump on parts of the body, indigestion, trouble swallowing, changes in warts or moles, change in new skin and nagging cough or hoarseness.

[00901] *Synthetic:* The term "synthetic" means produced, prepared, and/or manufactured by the hand of man. Synthesis of polynucleotides or polypeptides or other molecules of the present invention may be chemical or enzymatic.

[00902] *Targeted Cells:* As used herein, "targeted cells" refers to any one or more cells of interest. The cells may be found *in vitro, in vivo, in situ* or in the tissue or organ of an organism. The organism may be an animal, preferably a mammal, more preferably a human and most preferably a patient.

[00903] *Therapeutic Agent:* The term "therapeutic agent" refers to any agent that, when administered to a subject, has a therapeutic, diagnostic, and/or prophylactic effect and/or elicits a desired biological and/or pharmacological effect.

[00904] *Therapeutically effective amount:* As used herein, the term "therapeutically effective amount" means an amount of an agent to be delivered *{e.g.,* nucleic acid, drug, therapeutic agent, diagnostic agent, prophylactic agent, *etc.)* that is sufficient, when administered to a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[00905] *Therapeutically effective outcome:* As used herein, the term "therapeutically effective outcome" means an outcome that is sufficient in a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[00906] *Total daily dose:* As used herein, a "total daily dose" is an amount given or prescribed in 24 hr period. It may be administered as a single unit dose.

[00907] *Transcription factor:* As used herein, the term "transcription factor" refers to a DNA-binding protein that regulates transcription of DNA into RNA, for example, by activation or repression of transcription. Some transcription factors effect regulation of transcription alone, while others act in concert with other proteins. Some transcription factor can both activate and repress transcription under certain conditions. In general, transcription factors bind a specific target sequence or sequences highly similar to a specific consensus sequence in a regulatory region of a target gene. Transcription factors may regulate transcription of a target gene alone or in a complex with other molecules.

[00908] *Treating:* As used herein, the term "treating" refers to partially or completely alleviating, ameliorating, improving, relieving, delaying onset of, inhibiting progression of, reducing severity of, and/or reducing incidence of one or more symptoms or features of a particular infection, disease, disorder, and/or condition. For example, "treating" cancer may refer to inhibiting survival, growth, and/or spread of a tumor. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition and/or to a subject who exhibits only early signs of a disease, disorder, and/or condition for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[00909] *Tumor*. As used herein, a "tumor" is an abnormal growth of tissue, whether benign or malignant.

[00910] *Tumor growth:* As used herein, the term "tumor growth" or "tumor metastases" means an increased mass or volume of the tumor or expansion of the tumor distribution.

[00911] *Unmodified:* As used herein, "unmodified" refers to any substance, compound or molecule prior to being changed in any way. Unmodified may, but does not always, refer to the wild type or native form of a biomolecule. Molecules may undergo a series of modifications whereby each modified molecule may serve as the "unmodified" starting molecule for a subsequent modification.

Equivalents and Scope

[00912] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[00913] In the claims, articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a

given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[00914] It is also noted that the term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the term "consisting of is thus also encompassed and disclosed.

[00915] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[00916] In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the invention *(e.g.,* any nucleic acid or protein encoded thereby; any method of production; any method of use; *etc.)* can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[00917] All cited sources, for example, references, publications, databases, database entries, and art cited herein, are incorporated into this application by reference, even if not expressly stated in the citation. In case of conflicting statements of a cited source and the instant application, the statement in the instant application shall control.

[00918] Section and table headings are not intended to be limiting.

EXAMPLES

Example 1. Signal-sensor polynucleotide Production

[00919] Modified signal-sensor mRNAs (mmRNA) according to the invention may be made using standard laboratory methods and materials. The open reading frame (ORF) of the gene of interest may be flanked by a 5' untranslated region (UTR) which may contain

a strong Kozak translational initiation signal and/or an alpha-globin 3' UTR which may include an oligo(dT) sequence for templated addition of a poly-A tail. The modified mRNAs may be modified to reduce the cellular innate immune response. The modifications to reduce the cellular response may include pseudouridine (ψ) and 5-methyl-cytidine (5meC, 5mc or m^5C). (See, Kariko K et al. Immunity 23:165-75 (2005), Kariko K et al. Mol Ther 16:1833-40 (2008), Anderson BR et al. NAR (2010); herein incorporated by reference).

[00920] The ORF may also include various upstream or downstream additions (such as, but not limited to, β-globin, tags, etc.) may be ordered from an optimization service such as, but limited to, DNA2.0 (Menlo Park, CA) and may contain multiple cloning sites which may have Xbal recognition. Upon receipt of the construct, it may be reconstituted and transformed into chemically competent *E. coli.*

[00921] For the present invention, NEB DH5-alpha Competent *E. coli* may be used. Transformations are performed according to NEB instructions using 100 ng of plasmid. The protocol is as follows:

[00922] Thaw a tube of NEB 5-alpha Competent *E. coli* cells on ice for 10 minutes.

[00923] Add 1-5 μï containing 1 pg-100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. Do not vortex.

[00924] Place the mixture on ice for 30 minutes. Do not mix.

[00925] Heat shock at 42°C for exactly 30 seconds. Do not mix.

[00926] Place on ice for 5 minutes. Do not mix.

[00927] Pipette 950 μï of room temperature SOC into the mixture.

[00928] Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.

[00929] Warm selection plates to 37°C.

[00930] Mix the cells thoroughly by flicking the tube and inverting.

[00931] Spread 50-100 μï of each dilution onto a selection plate and incubate overnight at 37°C. Alternatively, incubate at 30°C for 24-36 hours or 25°C for 48 hours.

[00932] A single colony is then used to inoculate 5 ml of LB growth media using the appropriate antibiotic and then allowed to grow (250 RPM, 37° C) for 5 hours. This is then used to inoculate a 200 ml culture medium and allowed to grow overnight under the same conditions.

[00933] To isolate the plasmid (up to 850 μg), a maxi prep is performed using the Invitrogen PURELINK™ HiPure Maxiprep Kit (Carlsbad, CA), following the manufacturer's instructions.

[00934] In order to generate cDNA for *In Vitro* Transcription (IVT), the plasmid is first linearized using a restriction enzyme such as Xbal. A typical restriction digest with Xbal will comprise the following: Plasmid 1.0 μg; lOx Buffer 1.0 μï; Xbal 1.5 μï; dH_20 up to 10 μï; incubated at 37° C for 1 hr. If performing at lab scale (< 5μg), the reaction is cleaned up using Invitrogen's PURELINK™ PCR Micro Kit (Carlsbad, CA) per manufacturer's instructions. Larger scale purifications may need to be done with a product that has a larger load capacity such as Invitrogen's standard PURELINK™ PCR Kit (Carlsbad, CA). Following the cleanup, the linearized vector is quantified using the NanoDrop and analyzed to confirm linearization using agarose gel electrophoresis.

[00935] As a non-limiting example, G-CSF may represent the polypeptide of interest. Sequences used in the steps outlined in Examples 1-5 are shown in Table 12. It should be noted that the start codon (ATG) has been underlined in each sequence of Table 12.

Table 12. G-CSF Sequences

SEQ ID NO	Description
6592	cDNAsequence: ATGGCTGGACCTGCCACCCAGAGCCCCATGAAGCTGATGGCCCTGCAGCT GCTGCTGTGGCACAGTGCACTCTGGACAGTGCAGGAAGCCACCCCCCTGG GCCCTGCCAGCTCCCTGCCCCAGAGCTTCCTGCTCAAGTGCTTAGAGCAA GTGAGGAAGATCCAGGGCGATGGCGCAGCGCTCCAGGAGAAGCTGTGTG CCACCTACAAGCTGTGCCACCCCGAGGAGCTGGTGCTGCTCGGACACTCT CTGGGCATCCCCTGGGCTCCCCTGAGCAGCTGCCCCAGCCAGGCCCTGCA GCTGGCAGGCTGCTTGAGCCAACTCCATAGCGGCCTTTTCCTCTACCAGG GGCTCCTGCAGGCCCTGGAAGGGATCTCCCCCGAGTTGGGTCCCACCTTG GACACACTGCAGCTGGACGTCGCCGACTTTGCCACCACCATCTGGCAGCA GATGGAAGAACTGGGAATGGCCCCTGCCCTGCAGCCCACCCAGGGTGCC ATGCCGGCCTTCGCCTCTGCTTTCCAGCGCCGGGCAGGAGGGGTCCTGGT TGCCTCCCATCTGCAGAGCTTCCTGGAGGTGTCGTACCGCGTTCTACGCC ACCTTGCCCAGCCCTGA
6593	cDNA having T7 polymerase site, AfeI and Xba restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC ATGGCTGGACCTGCCACCCAGAGCCCCATGAAGCTGATGGCCCTGCAGCT GCTGCTGTGGCACAGTGCACTCTGGACAGTGCAGGAAGCCACCCCCCTGG GCCCTGCCAGCTCCCTGCCCCAGAGCTTCCTGCTCAAGTGCTTAGAGCAA

	GTGAGGAAGATCCAGGGCGATGGCGCAGCGCTCCAGGAGAAGCTGTGTG CCACCTACAAGCTGTGCCACCCCGAGGAGCTGGTGCTGCTCGGACACTCT CTGGGCATCCCCTGGGCTCCCCTGAGCAGCTGCCCCAGCCAGGCCCTGCA GCTGGCAGGCTGCTTGAGCCAACTCCATAGCGGCCTTTTCCTCTACCAGG GGCTCCTGCAGGCCCTGGAAGGGATCTCCCCCGAGTTGGGTCCCACCTTG GACACACTGCAGCTGGACGTCGCCGACTTTGCCACCACCATCTGGCAGCA GATGGAAGAACTGGGAATGGCCCCTGCCCTGCAGCCCACCCAGGGTGCC ATGCCGGCCTTCGCCTCTGCTTTCCAGCGCCGGGCAGGAGGGGTCCTGGT TGCCTCCCATCTGCAGAGCTTCCTGGAGGTGTCGTACCGCGTTCTACGCC ACCTTGCCCAGCCCTGA AGCGCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCC TTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGC CGCTCGAGCATGCATCTAGA
6594	Optimized sequence; containing T7 polymerase site, AfeI and XbaI restriction site TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC ATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGCCCTGCAGTT GCTGCTTTGGCACTCGGCCCTCTGGACAGTCCAAGAAGCGACTCCTCTCG GACCTGCCTCATCGTTGCCGCAGTCATTCCTTTTGAAGTGTCTGGAGCAG GTGCGAAAGATTCAGGGCGATGGAGCCGCACTCCAAGAGAAGCTCTGCG CGACATACAAACTTTGCCATCCCGAGGAGCTCGTACTGCTCGGGCACAGC TTGGGGATTCCCTGGGCTCCTCTCTCGTCCTGTCCGTCGCAGGCTTTGCAG TTGGCAGGGTGCCTTTCCCAGCTCCACTCCGGTTTGTTCTTGTATCAGGGA CTGCTGCAAGCCCTTGAGGGAATCTCGCCAGAATTGGGCCCGACGCTGGA CACGTTGCAGCTCGACGTGGCGGATTTCGCAACAACCATCTGGCAGCAGA TGGAGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGGCAAT GCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGTGGAGTCCTCGTAG CGAGCCACCTTCAATCATTTTTGGAAGTCTCGTACCGGGTGCTGAGACAT CTTGCGCAGCCGTGA AGCGCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCC TTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGC CGCTCGAGCATGCATCTAGA
6595	mRNA sequence (transcribed) GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACC AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGGCCCUGCAG UUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAAGAAGCGACUCCU CUCGGACCUGCCUCAUCGUUGCCGCAGUCAUUCCUUUUGAAGUGUCUG GAGCAGGUGCGAAAGAUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAG CUCUGCGCGACAUACAAACUUUGCCAUCCCGAGGAGCUCGUACUGCUC GGGCACAGCUUGGGGAUUCCCUGGGCUCCUCUCUCGUCCUGUCCGUCG CAGGCUUUGCAGUUGGCAGGGUGCCUUUCCCAGCUCCACUCCGGUUUG UUCUUGUAUCAGGGACUGCUGCAAGCCCUUGAGGGAAUCUCGCCAGAA UUGGGCCCGACGCUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCA ACAACCAUCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCUG CAGCCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCGUUUCAGCGC

	AGGGCGGGUGGAGUCCUCGUAGCGAGCCACCUUCAAUCAUUUUUGGAA GUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCCGUGA AGCGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCUC CCUUGCACCUGUACCUCUUGGUCUUUGAAUAAAGCCUGAGUAGGAAG

Example 2: PCR for cDNA Production

[00936] PCR procedures for the preparation of cDNA are performed using 2x KAPA HIFI™ HotStart ReadyMix by Kapa Biosystems (Woburn, MA). This system includes 2x KAPA ReadyMixl2.5 μΐ; Forward Primer (10 uM) 0.75 μΐ; Reverse Primer (10 uM) 0.75 μΐ; Template cDNA 100 ng; and dH_20 diluted to 25.0 μΐ. The reaction conditions are at 95° C for 5 min. and 25 cycles of 98° C for 20 sec, then 58° C for 15 sec, then 72° C for 45 sec, then 72° C for 5 min. then 4° C to termination.

[00937] The reverse primer of the instant invention incorporates a poly-Ti_{20} for a poly-Ai2o in the mRNA. Other reverse primers with longer or shorter poly(T) tracts can be used to adjust the length of the poly(A) tail in the mRNA.

[00938] The reaction is cleaned up using Invitrogen's PURELINK™ PCR Micro Kit (Carlsbad, CA) per manufacturer's instructions (up to 5 μg). Larger reactions will require a cleanup using a product with a larger capacity. Following the cleanup, the cDNA is quantified using the NanoDrop and analyzed by agarose gel electrophoresis to confirm the cDNA is the expected size. The cDNA is then submitted for sequencing analysis before proceeding to the *in vitro* transcription reaction.

Example 3. *In vitro* Transcription (IVT)

[00939] The *in vitro* transcription reaction generates mRNA containing modified nucleotides or modified RNA. The input nucleotide triphosphate (NTP) mix is made in-house using natural and un-natural NTPs.

[00940] A typical *in vitro* transcription reaction includes the following:

[00941] Template cDNA 1.0 μg

[00942] lOx transcription buffer (400 mM Tris-HCl pH 8.0, 190 mM $MgCl_2$, 50 mM DTT, 10 mM Spermidine) 2.0 μΐ

[00943] Custom NTPs (25mM each) 7.2 μΐ

[00944] RNase Inhibitor 20 U

[00945] T7 RNA polymerase 3000 U

[00946] dH_20 Up to 20.0 μΐ. and

[00947] Incubation at 37° C for 3 hr-5 hrs.

[00948] The crude IVT mix may be stored at 4° C overnight for cleanup the next day. 1 U of RNase-free DNase is then used to digest the original template. After 15 minutes of incubation at 37° C, the mRNA is purified using Ambion's MEGACLEAR™ Kit (Austin, TX) following the manufacturer's instructions. This kit can purify up to 500 μg of RNA. Following the cleanup, the RNA is quantified using the NanoDrop and analyzed by agarose gel electrophoresis to confirm the RNA is the proper size and that no degradation of the RNA has occurred.

Example 4. Enzymatic Capping of mRNA

[00949] Capping of the mRNA is performed as follows where the mixture includes: IVT RNA 60 μg-180 μg and dH_20 up to 72 μΐ. The mixture is incubated at 65° C for 5 minutes to denature RNA, and then is transferred immediately to ice.

[00950] The protocol then involves the mixing of lOx Capping Buffer (0.5 M Tris-HCl (pH 8.0), 60 mM KC1, 12.5 mM $MgCl_2$) (10.0 μΐ); 20 mM GTP (5.0 μΐ); 20 mM S-Adenosyl Methionine (2.5 μΐ); RNase Inhibitor (100 U); 2'-0-Methyltransferase (400U); Vaccinia capping enzyme (Guanylyl transferase) (40 U); dH_20 (Up to 28 μΐ); and incubation at 37° C for 30 minutes for 60 μg RNA or up to 2 hours for 180 μg of RNA.

[00951] The mRNA is then purified using Ambion's MEGACLEAR™ Kit (Austin, TX) following the manufacturer's instructions. Following the cleanup, the RNA is quantified using the NANODROP™ (ThermoFisher, Waltham, MA) and analyzed by agarose gel electrophoresis to confirm the RNA is the proper size and that no degradation of the RNA has occurred. The RNA product may also be sequenced by running a reverse-transcription-PCR to generate the cDNA for sequencing.

Example 5. PolyA Tailing Reaction

[00952] Without a poly-T in the cDNA, a poly-A tailing reaction must be performed before cleaning the final product. This is done by mixing Capped IVT RNA (100 μΐ); RNase Inhibitor (20 U); lOx Tailing Buffer (0.5 M Tris-HCl (pH 8.0), 2.5 M NaCl, 100 mM $MgCl_2$)(12.0 μΐ); 20 mM ATP (6.0 μΐ); Poly-A Polymerase (20 U); dH_20 up to 123.5 μΐ and incubation at 37° C for 30 min. If the poly-A tail is already in the transcript, then the tailing reaction may be skipped and proceed directly to cleanup with Ambion's

MEGACLEAR™ kit (Austin, TX) (up to 500 μg). Poly-A Polymerase is preferably a recombinant enzyme expressed in yeast.

[00953] For studies performed and described herein, the poly-A tail is encoded in the IVT template to comprise 160 nucleotides in length. However, it should be understood that the processivity or integrity of the polyA tailing reaction may not always result in exactly 160 nucleotides. Hence polyA tails of approximately 160 nucleotides, e.g, about 150-165, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164 or 165 are within the scope of the invention.

Example 6. Natural 5′ Caps and 5′ Cap Analogues

[00954] 5'-capping of modified RNA may be completed concomitantly during the *in* vzYro-transcription reaction using the following chemical RNA cap analogs to generate the 5'-guanosine cap structure according to manufacturer protocols: 3′-O-Me-m7G(5')ppp(5') G [the ARCA cap];G(5')ppp(5')A; G(5')ppp(5')G; m7G(5')ppp(5')A; m7G(5')ppp(5')G (New England BioLabs, Ipswich, MA). 5'-capping of modified RNA may be completed post-transcriptionally using a Vaccinia Virus Capping Enzyme to generate the "Cap 0" structure: m7G(5')ppp(5')G (New England BioLabs, Ipswich, MA). Cap 1 structure may be generated using both Vaccinia Virus Capping Enzyme and a 2'-0 methyl-transferase to generate: m7G(5')ppp(5')G-2'-0-methyl. Cap 2 structure may be generated from the Cap 1 structure followed by the 2'-0-methylation of the 5'-antepenultimate nucleotide using a 2'-0 methyl-transferase. Cap 3 structure may be generated from the Cap 2 structure followed by the 2'-0-methylation of the 5'-preantepenultimate nucleotide using a 2'-0 methyl-transferase. Enzymes are preferably derived from a recombinant source.

[00955] When transfected into mammalian cells, the modified mRNAs have a stability of between 12-18 hours or more than 18 hours, e.g., 24, 36, 48, 60, 72 or greater than 72 hours.

Example 7. Capping

A. Protein Expression Assay

[00956] Synthetic mRNAs encoding human G-CSF (cDNA shown in SEQ ID NO: 6567; mRNA sequence fully modified with 5-methylcytidine at each cytidine and pseudouridine replacement at each uridine site shown in SEQ ID NO: 6570 with a polyA

tail approximately 160 nucletodies in length not shown in sequence) containing the ARCA (3' 0-Me-m7G(5')ppp(5')G) cap analog or the Capl structure can be transfected into human primary keratinocytes at equal concentrations. 6, 12, 24 and 36 hours post-transfection the amount of G-CSF secreted into the culture medium can be assayed by ELISA. Synthetic mRNAs that secrete higher levels of G-CSF into the medium would correspond to a synthetic mRNA with a higher translationally-competent Cap structure.

B. Purity Analysis Synthesis

[00957] Synthetic mRNAs encoding human G-CSF (cDNA shown in SEQ ID NO: 6567; mRNA sequence fully modified with 5-methylcytidine at each cytidine and pseudouridine replacement at each uridine site shown in SEQ ID NO: 6570 with a polyA tail approximately 160 nucletodies in length not shown in sequence) containing the ARCA cap analog or the Capl structure crude synthesis products can be compared for purity using denaturing Agarose-Urea gel electrophoresis or HPLC analysis. Synthetic mRNAs with a single, consolidated band by electrophoresis correspond to the higher purity product compared to a synthetic mRNA with multiple bands or streaking bands. Synthetic mRNAs with a single HPLC peak would also correspond to a higher purity product. The capping reaction with a higher efficiency would provide a more pure mRNA population.

C. Cytokine Analysis

[00958] Synthetic mRNAs encoding human G-CSF (cDNA shown in SEQ ID NO: 6567; mRNA sequence fully modified with 5-methylcytidine at each cytidine and pseudouridine replacement at each uridine site shown in SEQ ID NO: 6570 with a polyA tail approximately 160 nucletodies in length not shown in sequence) containing the ARCA cap analog or the Capl structure can be transfected into human primary keratinocytes at multiple concentrations. 6, 12, 24 and 36 hours post-transfection the amount of pro-inflammatory cytokines such as TNF-alpha and IFN-beta secreted into the culture medium can be assayed by ELISA. Synthetic mRNAs that secrete higher levels of pro-inflammatory cytokines into the medium would correspond to a synthetic mRNA containing an immune-activating cap structure.

D. Capping Reaction Efficiency

[00959] Synthetic mRNAs encoding human G-CSF (cDNA shown in SEQ ID NO: 6567; mPvNA sequence fully modified with 5-methylcytidine at each cytidine and pseudouridine replacement at each uridine site shown in SEQ ID NO: 6570 with a polyA tail approximately 160 nucletodies in length not shown in sequence) containing the ARCA cap analog or the Capl structure can be analyzed for capping reaction efficiency by LC-MS after capped mRNA nuclease treatment. Nuclease treatment of capped mRNAs would yield a mixture of free nucleotides and the capped 5'-5-triphosphate cap structure detectable by LC-MS. The amount of capped product on the LC-MS spectra can be expressed as a percent of total mRNA from the reaction and would correspond to capping reaction efficiency. The cap structure with higher capping reaction efficiency would have a higher amount of capped product by LC-MS.

Example 8. Agarose Gel Electrophoresis of Modified RNA or RT PCR Products

[00960] Individual modified RNAs (200-400 ng in a 20 μï volume) or reverse transcribed PCR products (200-400 ng) are loaded into a well on a non-denaturing 1.2% Agarose E-Gel (Invitrogen, Carlsbad, CA) and run for 12-15 minutes according to the manufacturer protocol.

Example 9. Nanodrop Modified RNA Quantification and UV Spectral Data

[00961] Modified RNAs in TE buffer (1 μï) are used for Nanodrop UV absorbance readings to quantitate the yield of each modified RNA from an *in vitro* transcription reaction.

Example 10. Formulation of signal-sensor polynucleotides

[00962] Signal-sensor polynucleotides may be formulated for *in vitro* and *in vivo* experiments according to the methods taught in International Application PCT/US 12/069610 filed December 14, 2012, the contents of which are incorporated herein by reference in their entirety.

Example 11. Assays and methods of detection or analysis of signal-sensor polynucleotides.

[00963] Signal-sensor polynucleotides may be investigated using the methods described in co-pending International Patent application No. PCT/US20 13/030070 filed

March 9, 2013 and US Patent Application No. US 61/681,742 filed August 10, 2012 (MNC2), the contents of which are incorporated herein by reference in their entirety.

Example 12. Cell lines for the study of Signal-Sensor Polynucleotides

[00964] Signal-sensor polynucleotides may be investigated in any number of cancer or normal cell lines. Cell lines useful in the present invention include those from ATCC (Manassas, VA) and are listed in Table 13.

Table 13. Cell lines

ATCC Number	Hybridoma or Cell line Description	Name
CCL-171	Homo sapiens (human) Source: Organ: lung Disease: normal Cell Type: fibroblast	MRC-5
CCL-185	Homo sapiens (human) Source: Organ: lung Disease: carcinoma	A549
CCL-248	Homo sapiens (human) Source: Organ: colon Disease: colorectal carcinoma Derived from metastatic site: lung	T84
CCL-256	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H2126 [H2126]
CCL-257	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; classic small cell lung cancer	NCI-H1688 [H1688]
CCL-75	Homo sapiens (human) Source: Organ: lung Disease: normal Cell Type: fibroblast	WI-38
CCL-75.1	Homo sapiens (human) Source: Organ: lung Cell Type: fibroblastSV40 transformed	WI-38 VA-13 subline 2RA
CCL-95.1	Homo sapiens (human) Source: Organ: lung Cell Type: SV40 transformed	WI-26 VA4
CRL- 10741	Homo sapiens (human) Source: Organ: liver Disease: hepatocellular carcinoma	C3A [HepG2/C3A, derivative of Hep G2 (ATCC HB-8065)]
CRL- 11233	Homo sapiens (human) Source: Organ: liver Tissue: left lobe Cell Type: epithelialimmortalized with SV40 large T antigen	THLE-3
CRL- 11351	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer; multidrug resistant Cell Type: epithelial	H69AR
CRL- 1848	Homo sapiens (human) Source: Organ: lung Disease: mucoepidermoid pulmonary carcinoma	NCI-H292 [H292]
CRL-1918	Homo sapiens (human) Source: Organ: pancreas	CFPAC-1

	Disease: ductal adenocarcinoma; cystic fibrosis Derived from metastatic site: liver metastasis	
CRL-1973	Homo sapiens (human) Source: Organ: testis Disease: malignant pluripotent embryonal carcinoma Derived from metastatic site: lung	NTERA-2 cl.D1 [NT2/D1]
CRL-2049	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer	DMS 79
CRL-2062	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer	DMS 53
CRL-2064	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: liver	DMS 153
CRL-2066	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer	DMS 114
CRL-2081	Homo sapiens (human) Source: Disease: biphasic mesothelioma Derived from metastatic site: lung	MSTO-211H
CRL-2170	Homo sapiens (human) Source: Organ: lung Disease: alveolar cell carcinoma	SW 1573 [SW-1573, SW1573]
CRL-2177	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer	SW 1271 [SW-1271, SW1271]
CRL-2195	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Cell Type: large cell, variant;	SHP-77
CRL-2233	Homo sapiens (human) Source: Organ: liver Disease: hepatocellular carcinoma	SNU-398
CRL-2234	Homo sapiens (human) Source: Organ: liver Tumor Stage: grade II-III/IV Disease: hepatocellular carcinoma	SNU-449
CRL-2235	Homo sapiens (human) Source: Organ: liver Tumor Stage: grade III/IV Disease: hepatocellular carcinoma	SNU-182
CRL-2236	Homo sapiens (human) Source: Organ: liver Tumor Stage: grade II-IV/V Disease: hepatocellular carcinoma	SNU-475
CRL-2237	Homo sapiens (human) Source: Organ: liver Tumor Stage: grade IV/V Disease: pleomorphic hepatocellular carcinoma	SNU-387
CRL-2238	Homo sapiens (human) Source: Organ: liver Tumor Stage: grade III/IV Disease: pleomorphic hepatocellular carcinoma	SNU-423
CRL-2503	Homo sapiens (human) Source: Organ: lung Tissue: bronchus Disease: normal	NL20
CRL-2504	Homo sapiens (human) Source: Organ: lung Tissue: bronchus Disease: normal	NL20-TA [NL20T-A]
CRL-2706	Homo sapiens (human) Source: Organ: liver	THLE-2

	Tissue: left lobe Cell Type: epithelialSV40 transformed	
CRL-2741	Homo sapiens (human) Source: Organ: lung Tissue: bronchus Cell Type: epithelialHPV- 16 E6/E7 transformed	HBE135-E6E7
CRL-2868	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma Cell Type: epithelial	HCC827
CRL-2871	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma Derived from metastatic site: pleural effusion Cell Type: epithelial	HCC4006
CRL-5800	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H23 [H23]
CRL-5803	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1299
CRL-5804	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H187 [HI 87]
CRL-5807	Homo sapiens (human) Source: Organ: lung Tissue: bronchiole; alveolus Disease: bronchioalveolar carcinoma; non-small cell lung cancer	NCI-H358 [H-358, H358]
CRL-5808	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H378 [H378]
CRL-5810	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 2 Disease: adenocarcinoma; non-small cell lung cancer	NCI-H522 [H522]
CRL-581 1	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; variant small cell lung cancer Derived from metastatic site: bone marrow	NCI-H526 [H526]
CRL-5815	Homo sapiens (human) Source: Organ: lung Tissue: bronchus Disease: carcinoid	NCI-H727 [H727]
CRL-5816	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 2 Disease: carcinoma; non-small cell lung cancer	NCI-H810 [H810]
CRL-5817	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	NCI-H889 [H889]
CRL-5818	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1155 [HI 155]

CRL-5819	Homo sapiens (human) Source: Organ: lung Disease: papillary adenocarcinoma Derived from metastatic site: lymph node	NCI-H1404 [HI 404]
CRL-5822	Homo sapiens (human) Source: Organ: stomach Disease: gastric carcinoma Derived from metastatic site: liver	NCI-N87 [N87]
CRL-5823	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; variant small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H196 [HI 96]
CRL-5824	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H21 1 [H21 1]
CRL-5825	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H220 [H220]
CRL-5828	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: brain	NCI-H250 [H250]
CRL-5831	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; variant small cell lung cancer Derived from metastatic site: lymph node	NCI-H524 [H524]
CRL-5834	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3A Disease: adenosquamous carcinoma; non-small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H647 [H647]
CRL-5835	Homo sapiens (human) Source: Organ: lung Disease: bronchioalveolar carcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H650 [H650]
CRL-5836	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: bone marrow	NCI-H71 1 [H71 1]
CRL-5837	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: bone marrow	NCI-H719 [H719]
CRL-5840	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	NCI-H740 [H740]
CRL-5841	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E	NCI-H748 [H748]

	Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	
CRL-5842	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: soft tissue	NCI-H774 [H774]
CRL-5844	Homo sapiens (human) Source: Organ: lung Tumor stage: 3B Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H838 [H838]
CRL-5845	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; variant small cell lung cancer Derived from metastatic site: lymph node	NCI-H841 [H841]
CRL-5846	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H847 [H847]
CRL-5849	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H865 [H865]
CRL-5850	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H920 [H920]
CRL-5853	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H1048 [H1048]
CRL-5855	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: bone marrow	NCI-H1092 [HI 092]
CRL-5856	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	NCI-H1105 [HI 105]
CRL-5858	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; small cell lung cancer Derived from metastatic site: lymph node	NCI-H1184 [HI 184]
CRL-5859	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H1238 [H1238]
CRL-5864	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: cervix	NCI-H1341 [H1341]

CRL-5867	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3A Disease: carcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1385 [H1385]
CRL-5869	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer	NCI-H1417 [H1417]
CRL-5870	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1435 [H1435]
CRL-5871	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	NCI-H1436 [H1436]
CRL-5872	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 1 Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H1437 [H1437]
CRL-5874	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H1522 [HI 522]
CRL-5875	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1563 [H1563]
CRL-5876	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1568 [H1568]
CRL-5877	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: adenocarcinoma Derived from metastatic site: soft tissue	NCI-H1573 [H1573]
CRL-5878	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: non-small cell lung cancer Cell Type: large cell;	NCI-H1581 [H1581]
CRL-5879	Homo sapiens (human) Source: Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H1618 [H1618]
CRL-5881	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3B Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1623 [HI 623]
CRL-5883	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3B Disease: adenocarcinoma; bronchoalveolar carcinoma Derived from metastatic site: pleural effusion	NCI-H1650 [H-1650, H1650]
CRL-5884	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1651 [H1651]
CRL-5885	Homo sapiens (human) Source: Organ: lung	NCI-H1666 [H-

	Disease: adenocarcinoma; bronchoalveolar carcinoma Derived from metastatic site: pleural effusion	1666, H1666]
CRL-5886	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; classic small cell lung cancer	NCI-H1672 [HI 672]
CRL-5887	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3B Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1693 [H1693]
CRL-5888	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: ascites	NCI-H1694 [HI 694]
CRL-5889	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 1 Disease: non-small cell lung cancer Cell Type: squamous cell;	NCI-H1703 [H1703]
CRL-5891	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1734 [H-1734, H1734]
CRL-5892	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: liver	NCI-H1755 [H1755]
CRL-5892	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: liver	NCI-H1755 [H1755]
CRL-5893	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: carcinoma; non-small cell lung cancer Derived from metastatic site: lymph node Cell Type: neuroendocrine;	NCI-H1770 [HI 770]
CRL-5896	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1793 [H1793]
CRL-5898	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; classic small cell lung cancer	NCI-H1836 [H1836]
CRL-5899	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1838 [H1838]
CRL-5900	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: non-small cell lung cancer Derived from metastatic site: pleural effusion Cell Type: squamous cell;	NCI-H1869 [HI 869]
CRL-5902	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	NCI-H1876 [HI 876]

CRL-5903	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H1882 [H1882]
CRL-5904	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: poorly differentiated carcinoma; non-small cell lung cancer Derived from metastatic site: brain Cell Type: large cell;	NCI-H1915 [H1915]
CRL-5906	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: lymph node	NCI-H1930 [H1930]
CRL-5907	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3B Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: soft tissue	NCI-H1944 [HI 944]
CRL-5908	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H1975 [H-1975, H1975]
CRL-5909	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3A Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H1993 [H1993]
CRL-5912	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3A Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H2023 [H2023]
CRL-5913	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: lymph node	NCI-H2029 [H2029]
CRL-5914	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H2030 [H2030]
CRL-5917	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 1 Disease: mixed; small cell lung cancer; adenocarcinoma; squamous cell carcinoma	NCI-H2066 [H2066]
CRL-5918	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3A Disease: adenocarcinoma; non-small cell lung cancer	NCI-H2073 [H2073]
CRL-5920	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; classic small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H2081 [H2081]
CRL-5921	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H2085 [H2085]

CRL-5922	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 1 Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H2087 [H2087]
CRL-5923	Homo sapiens (human) Source: Organ: lung Tissue: neuroendocrine Tumor Stage: stage 4 Disease: non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H2106 [H2106]
CRL-5924	Homo sapiens (human) Source: Organ: lung Disease: non-small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H21 10 [H21 10]
CRL-5926	Homo sapiens (human) Source: Organ: lung Disease: non-small cell lung cancer	NCI-H2135 [H2135]
CRL-5927	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: lymph node	NCI-H2141 [H2141]
CRL-5929	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H2171 [H2171]
CRL-5930	Homo sapiens (human) Source: Organ: lung Disease: non-small cell lung cancer	NCI-H2172 [H2172]
CRL-5931	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H2195 [H2195]
CRL-5932	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H2196 [H2196]
CRL-5933	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: lymph node	NCI-H2198 [H2198]
CRL-5934	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer	NCI-H2227 [H2227]
CRL-5935	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer	NCI-H2228 [H2228]
CRL-5938	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 1 Disease: mixed; small cell lung cancer; adenocarcinoma; squamous cell carcinoma	NCI-H2286 [H2286]
CRL-5939	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: lymph node	NCI-H2291 [H2291]

CRL-5940	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; small cell lung cancer Derived from metastatic site: lymph node	NCI-H2330 [H2330]
CRL-5941	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 3A Disease: adenocarcinoma; non-small cell lung cancer	NCI-H2342 [H2342]
CRL-5942	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 1 Disease: adenocarcinoma; non-small cell lung cancer	NCI-H2347 [H2347]
CRL-5944	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: ascites	NCI-H2405 [H2405]
CRL-5945	Homo sapiens (human) Source: Organ: lung Disease: non-small cell lung cancer	NCI-H2444 [H2444]
CRL-5975	Homo sapiens (human) Source: Organ: lung Disease: carcinoid	UMC-1 1
CRL-5976	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: lymph node	NCI-H64 [H64]
CRL-5978	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: liver	NCI-H735 [H735]
CRL-5978	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: liver	NCI-H735 [H735]
CRL-5982	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage L Disease: carcinoma; small cell lung cancer	NCI-H1963 [H1963]
CRL-5983	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H2107 [H2107]
CRL-5984	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage E Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H2108 [H2108]
CRL-5985	Homo sapiens (human) Source: Organ: lung Tumor Stage: stage 4 Disease: adenocarcinoma; non-small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H2122 [H2122]
CRL-7343	Homo sapiens (human) Source: Organ: lung Disease: cancer	Hs 573.T
CRL-7344	Homo sapiens (human) Source: Organ: lung	Hs 573.Lu

CRL-8024	Homo sapiens (human) Source: Organ: liver Disease: hepatoma Cell Type: Alexander cells;	PLC/PRF/5
CRL-9609	Homo sapiens (human) Source: Organ: lung Tissue: bronchus Disease: normal Cell Type: epithelialvirus transformed	BEAS-2B
HB-8065	Homo sapiens (human) Source: Organ: liver Disease: hepatocellular carcinoma	Hep G2
HTB-105	Homo sapiens (human) Source: Organ: testes Disease: embryonal carcinoma, malignant Derived from metastatic site: lung	Tera- 1
HTB-106	Homo sapiens (human) Source: Disease: malignant embryonal carcinoma Derived from metastatic site: lung	Tera-2
HTB-1 19	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer	NCI-H69 [H69]
HTB-120	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H128 [H128]
HTB-168	Homo sapiens (human) Source: Organ: lung Tissue: bronchus Disease: bronchogenic carcinoma	ChaGo-K-1
HTB-171	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H446 [H446]
HTB-172	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H209 [H209]
HTB-173	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H146 [HI 46]
HTB-174	Homo sapiens (human) Source: Organ: lung Disease: papillary adenocarcinoma	NCI-H441 [H441]
HTB-175	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: pleural effusion	NCI-H82 [H82]
HTB-177	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; large cell lung cancer Derived from metastatic site: pleural effusion	NCI-H460 [H460]
HTB-178	Homo sapiens (human) Source: Organ: lung Disease: adenosquamous carcinoma	NCI-H596 [H596]
HTB-179	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma Derived from metastatic site: pleural effusion	NCI-H676B [H676B]
HTB-180	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer Derived from metastatic site: bone marrow	NCI-H345 [H345]

HTB-181	Homo sapiens (human) Source: Organ: lung Disease: papillary adenocarcinoma Derived from metastatic site: lymph node	NCI-H820 [H820]
HTB-182	Homo sapiens (human) Source: Organ: lung Disease: squamous cell carcinoma	NCI-H520 [H520]
HTB-183	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; large cell lung cancer Derived from metastatic site: lymph node	NCI-H661 [H661]
HTB-184	Homo sapiens (human) Source: Organ: lung Disease: carcinoma; small cell lung cancer; extrapulmonary origin Derived from metastatic site: adrenal gland	NCI-H510A [H510A, NCI-IK 10]
HTB-52	Homo sapiens (human) Source: Organ: liver Tissue: ascites Disease: adenocarcinoma	SK-HEP-1
HTB-53	Homo sapiens (human) Source: Organ: lung Disease: carcinoma	A-427
HTB-54	Homo sapiens (human) Source: Organ: lung Tumor Stage: grade III Disease: epidermoid carcinoma Derived from metastatic site: pleura	Calu-1
HTB-55	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma Derived from metastatic site: pleural effusion	Calu-3
HTB-56	Homo sapiens (human) Source: Organ: unknown, probably lung Disease: anaplastic carcinoma	Calu-6
HTB-57	Homo sapiens (human) Source: Organ: lung Disease: adenocarcinoma	**SK**-LU-1
HTB-58	Homo sapiens (human) Source: Organ: lung Disease: squamous cell carcinoma Derived from metastatic site: pleural effusion	SK-MES-1
HTB-59	Homo sapiens (human) Source: Organ: lung Tumor Stage: grade IV Disease: squamous cell carcinoma	SW 900 [SW-900, SW900]
HTB-64	Homo sapiens (human) Source: Disease: malignant melanoma Derived from metastatic site: lung	Malme-3M
HTB-79	Homo sapiens (human) Source: Organ: pancreas Disease: adenocarcinoma Derived from metastatic site: liver	Capan- 1

Example 13. Animal models for the study of signal-sensor polynucleotides.

[00965] Various animal models are available for the study of the signal-sensor polynucleotides of the present invention. These include, among others, models for lung and liver cancers.

[00966] The lung cancer model of Fukazawa et al (Anticancer Research, 2010; 30: 4193-4200) is employed in studies of signal-sensor polynucleotides. Briefly, a congenic mouse is created by crossing a ubiquitously expressing dominant negative Myc (Omomyc) mouse with a KRAS mutation- positive lung cancer model mouse. In the presence of Omomyc, lung tumors caused by the expression of mutated KRAS regresses in the congenic mouse, indicating that Omomyc caused tumor cell death of KRAS mutation-positive lung cancer.

[00967] Human lung cancer xenografts are also prepared by the method of Fukazawa. Briefly, human lung cancer xenografts are established in 4-week-old female BALB/C nude mice (Charles River Laboratories Japan, Kanagawa, Japan) by subcutaneous inoculation of 4x106 A549 cells into the dorsal flank. The mice are randomly assigned into six groups (n=6/group). After the tumors reach a diameter of about 0.5 cm (approximately 6 days after tumor inoculations), each group of mice are injected with 100 µï solution containing PBS, 5x1010 vp of control or signal-sensor polynucleotide into the dorsalflank tumor for the selected dosing regimen. Animals are then observed closely and survival studiesor other analyses are performed.

[00968] The LSL-KRAS^{G12D}: TRE Omomyc:CMV rtTA triple transgenic model involves the use of an adenovirus expressing Cre recombinase which is administered via inhalation to induce oncogene expression via excision of the floxed STOP codon, and ubiquitous Omomyc expression is controlled via doxycycline. The model is reported in Soucek et al. (Nature, 1-5 (2008)). The mice of Soucek may be crossed with the LSLKRAS^{G12D} single transgenic mice (Jackson Laboratories) and may be used for inhalation delivered or otherwise lung-delivered studies of signal-sensor polynucleotides expressing MYC inhibitor **D** or other oncology related polypeptide.

[00969] Mouse-in-mouse models may also be employed. Such models are akin to the p53-/-:c-Myc overexpressing HCC model of Zender (Cell. 2006 June 30; 125(7): 1253-1267).

[00970] Design of such models involve starting with the WT or tumor suppressor deleted (such as p53 -/-) 129 Sv/Ev Mm ES cell clone; introduction of liver activated protein (LAP) promoter directed tetracycline transactivator (tTA) and tetO-luciferase for liver specific imaging; freezing the resulting LAP-tTA: tetO-luciferase clones to be used for c-Myc as well as other liver relevant programs oncogene; adding tetO driven oncogene, e.g. tetOcMyc; Freeze resulting LAP-tTA: tetO-luciferase: tetO-MYC clones; injecting resulting ES clones into C57B1/6 blastocytes and implant in pseudo pregnant mothers whereby the resulting chimeric animals are the tumor model upon removal of doxycycline (i.e. Tet- Off). The model will ideally evince inducible nodules of c-Myc-driven, luciferase-expressing HCC surrounded by normal hepatocytes.

[00971] Orthotopic HCC models using the HEP3B cell lines in mice may also be used (Crown Bio).

[00972] Nongermline genetically engineered mouse model (NGEMM) platform is a platform that could also be utilized for exploring signal-sensor polynucleotides.

Example 14. Inhibition of HIFl-alpha: SHARP1 and CITED4

[00973] Hypoxia-inducible factors (HIFs) control cellular adaptation to oxygen deprivation. Cancer cells engage HIFs to sustain their growth in adverse conditions, thus promoting a cellular reprograming that includes metabolism, proliferation, survival and mobility. HIFs overexpression in human cancer biopsies correlates with high metastasis and mortality.

[00974] Hifs regulate genes related to metabolism such as GLUT 1, GLUT3, ALDOA, ENOl, GAPDH, HKl, HK2, PFKL, PGKl, PKM2, LDHA , proliferation such as IGF-2, TGFA, VEGFA, survival such as TERT, NANOG, OCT4 and cell migration-invasion such as ZEB1, ZEB2, SNAI2, MMP14, MMP9, AMF, MET, PTHrP. (Keith , et al Nat Rev Cancer 2012; 12:9-22).

[00975] To investigate the destabilization of cancer, one or more signal-sensor polynucleotides may be administered to the cancer cell. The selection of the sequence, dose or administrative route is optionally informed by diagnostic evaluation of the cell, tumor, tissue or organism including, but not limited to, expression profiling of the cancer, metabolic evaluation (hypoxic, acidotic), apoptotic vs. survival profiling, cell cycle vs. senescent profiling, immune sensitivities, and/or evaluation of stromal factors.

[00976] In one arm of the study signal-sensor polynucleotides encoding either or both oncology related polypeptides, CITED4 and SHARP 1 are administered where administration of either or both results in the inhibition of the transcriptome of HIF-1alpha in cancer cells. Suppression of HIFl -alpha gene regulated expression occurs upon administration with higher suppression when both polynucleotides are administered together.Reporter constructs such as luciferase under HIFl -alpha are used in the manner similar to the methods disclosed in van de Sluis et al, (J Clin Invest. 2010; 120(6):21 19—2130). It is known that both CITED4 and SHARP 1 expression results in decreased HIF1-alpha and concomitant reduction in HIFl -alpha regulated gene expression. Evaluation of cell death and/or proliferation isalso performed.

[00977] Additional experiments involve the use a cancer cell line where CITED4 and SHARP 1 are themselves down regulated either under hypoxic conditions. Therefore a positive result demonstrates that specifically targeting the metabolic profile (in this case hypoxic-adaptations of CITED4 and SHAPR1) with replacement of native proteins via signal-sensor polynucleotides can directly impact the transcriptome and survival advantage of cancer cells with this profile. Further, the data would show that the relative impact of signal-sensor polynucleotide vs. vehicle under hypoxic conditions was more significant for cancer cells than for normal cells. (i.e., the cancer cells have a disproportionate survival advantage based on their CITED4+SHARP1 down regulation) that makes them more sensitive to the replacement of this protein then a normal cell is to overproduction of it. It is understood that a cancer cell will likely be experiencing hypoxic conditions and that a normal cell under normoxic conditions might be able to tolerate CITED4 and SHARP 1 over expression because the normal cell is not dependent on HIFl alpha transctiptome for survival advantage.

[00978] In vivo experiments are performed according to the design of the in vitro experiments where the animal model is one evincing metastasis in the cancer setting because HIF-1 alpha appears to confer the largest portion of its advantage in metastasis. Animals are administered the signal-sensor polynucleotide compared to no treatment or a control polynucleotide. Animal cells, tissues and/or organs are then evaluated for alterations in gene expression profiles or transcriptome levels.

Example_15. Alteration_of Signal-Sensor_Polynucleotide_trafficking:_NLS and NES

[00979] Two nuclear export signals (NES) which may be incorporated into the Signal-sensor polynucleotides of the present invention include those reported by Muller, et al (Traffic, 2009, 10: 514-527) and are associated with signaling via the gene COMMD1. These are NESl, PVAIIELEL (SEQ ID NO 6596) and NES2, VNQILKTLSE (SEQ ID NO 6597).

[00980] Nuclear localization signals may also be used. One such sequence is PKKKRKV (SEQ ID NO: 6598).

[00981] Cell lines or mice are administered one or more signal-sensor polynucleotides having a NLS or NES encoded therein. Upon administration the polynucleotide is trafficked to an alternate location, e.g., into the nucleus using the NLS. The oncology related polypeptide having the NLS would be trafficked to the nucleus where it would deliver either a survival or death signal to the nuclear microenvironment. Polypeptides which may be localized to the nucleus include those with altered binding properties for DNA which will function to alter the expression profile of the cell in a therapeutically benefical manner for the cell, tissue or organism.

[00982] In one experiment, the signal-sensor polynucleotide encodes a COMMD1 mutl/mut 2 + NLS (e.g., both NES signals disrupted plus a NLS added) following the methods of Muller et al, (Traffic 2009; 10: 514-527) and van de Sluis et al, (J Clin Invest. 2010;120 (6):21 19—2130). The signal sequence may encode an oncology related polypeptide or a scrambled sequence which is not translatable. The signal sequence encoded would interact with HIF1-alpha to alter the transcritome of the cancer cells.

[00983] The experiment is repeated under normal and hypoxic conditions.

[00984] Once identified the HIF1-alpha dependent signal-sensor polynucleotide is tested in cancer cell lines clonal survival or a marker of apoptosis is measured and compared to control or mock treated cells.

Example 16. Signal-sensor Polynucleotides in the treatment of Hepatocellular Carcinoma (HCC): disruption of cancer cell transcriptome.

[00985] Using the animal models outlined in Example 13, animals are treated with signal-sensor polynucleotide for MYC inhibitor D vs. negative control (untranslatable mRNA for MYC inhibitor D) vs. vehicle. For the KRas model addition of the existing transduced OmoMyc model may also be utilized. Animals are then evaluated for gene

expression, tumor status or for any of the hallmarks associated with cancer phenotypes or genotypes.

Example 17. Cytoprotective Signal-sensor polynucleotides

[00986] Deliver one or multiple mRNA therapeutics resulting in a protein (native or non-native, intracellular or extracellular) that confers a cytoprotective advantage to normal cells in the setting of cancer therapeutics (both mRNA and non-mRNA)

Example 18. miRNA binding sites (BS) useful as Sensor sequences in Signal-Sensor polynucleotides

[00987] miRNA-binding sites are used in the 3'UTR of mRNA therapeutics to direct cytotoxic or cytoprotective mRNA therapeutics to specific cells (normal and/or cancerous).

[00988] A strong apoptotic signal (i.e., AIFsh - Apoptosis Inducing Factor short isoform, constitutively active (C.A.) caspase 6 (also known as Rev-caspase-6) - is a a component of HSVl-tk - herpes simplex virus 1-thymidine kinase) is encoded as the oncology-related polypeptide or "signal" and is encoded along with a series of 3'UTR miR binding sites, such as that for mir-122a, that would make the signal-sensor polynucleotide relatively much more stable in cancerous cells than in normal cells.

[00989] Experiments comparing cancer vs. normal heaptic cell lines where the cancer cell lines have a specific miR signature are performed in vitro. SNU449 or HEP3B (human derived HCC cell lines) are used because both have been shown to have "undetectable miR-122a", whereas normal hepatocytes should have very high miR-122a levels.

A. AIFsh encoded polypeptide study

[00990] First a cancer cell is selected which is sensitive to AIFsh signal-sensor polynucleotide (i.e., it results in apoptosis).

[00991] Three miR-122a binding sites are encoded into the 3'UTR of an mRNA sequence for AIFsh and the study arms include 2 cell lines (normal hepatocyte, SNU449 or HEP3B) x 5 treatments (vehicle alone, signal-sensor polynucleotide untranslatabe, signal-sensor polynucleotide AIFsh (no miR BS in 3'UTR), 3'UTR[miR122a BS **x3]**-signal-sensor polynucleotide untranslatable, 3'UTR[miR122a BS x3]- signal-sensor polynucleotide AIFsh).

[00992] The expected result would be significant apoptosis in the face of signal-sensor polynucleotide AIFsh in both normal and cancer (HEP3B or SNU449) cell lines in the absence of any 3'UTR-miR122a BS. However, a significant difference in the relative apoptosis of normal vs. cancer cell lines in the face of 3'UTR [miR122a BS x3]- signal-sensor polynucleotide AIFsh.

[00993] Reversibility of the effect is shown with the co-administration of miR122a to the cancer cell line (e.g., through some transduction of the miR122a activity back into the cancer cell line).

B. C.A. Caspase 6 encoded polypeptide study

[00994] First a cancer cell is selected which is sensitive to C.A. caspase 6 signal-sensor polynucleotide (i.e., it results in apoptosis).

[00995] Three miR-122a binding sites are encoded into the 3'UTR of an mRNA sequence for C.A. caspase 6 and the study arms include 2 cell lines (normal hepatocyte, SNU449 or HEP3B) x 5 treatments (vehicle alone, signal-sensor polynucleotide untranslatabe, signal-sensor polynucleotide C.A. caspase 6 (no miR BS in 3'UTR), 3'UTR[miR122a BS x3]- signal-sensor polynucleotide untranslatable, 3'UTR[miR122a BS x3]- signal-sensor polynucleotide C.A. caspase 6).

[00996] The expected result would be significant apoptosis in the face of signal-sensor polynucleotide C.A. caspase 6 in both normal and cancer (HEP3B or SNU449) cell lines in the absence of any 3'UTR-miR122a BS. However, a significant difference in the relative apoptosis of normal vs. cancer cell lines in the face of 3'UTR [miR122a BS x3]- signal-sensor polynucleotide C.A. caspase 6 .

C. HSVl-tk encoded polypeptide study

[00997] First a cancer cell is selected which is sensitive to HSVl-tk signal-sensor polynucleotide (i.e., it results in apoptosis).

[00998] Three miR-122a binding sites are encoded into the 3'UTR of an mRNA sequence for HSVl-tk and the study arms include 2 cell lines (normal hepatocyte, SNU449 or HEP3B) x 5 treatments (vehicle alone, signal-sensor polynucleotide untranslatabe, signal-sensor polynucleotide HSVl-tk (no miR BS in 3'UTR), 3'UTR[miR122a BS x3]- signal-sensor polynucleotide untranslatable, 3'UTR[miR122a BS x3]- signal-sensor polynucleotide HSVl-tk).

[00999] The expected result would be significant apoptosis in the face of signal-sensor polynucleotide HSVl-tk in both normal and cancer (HEP3B or SNU449) cell lines in the absence of any 3'UTR-miR122a BS. However, a significant difference in the relative apoptosis of normal vs. cancer cell lines in the face of 3'UTR [miR122a BS x3]- signal-sensor polynucleotide HSVl-tk.

[001000] Reversibility of the effect is shown with the co-administration of miR122a to the cancer cell line (e.g., through some transduction of the miR122a activity back into the cancer cell line).

D. *In vivo* study of signal-sensor polynucleotides

[001001] *In vivo* animal studies are performed for AIFsh, C.A. caspase 6 and HSVl-tk using any of the models disclosed herein or a commercially available orthotopic HCC model.

Example 19. Expression of modified nucleic acid with microRNA binding site

[001002] Human embryonic kidney epithelial cells (HEK293A) and primary human hepatocytes (Hepatocytes) were seeded at a density of 200,000 per well in 500 ul cell culture medium (InVitro GRO medium from Celsis, Chicago, IL). G-CSF mRNA having an alpha-globin 3'UTR (G-CSF alpha) (mRNA sequence is shown in SEQ ID NO: 6599; polyA tail of approximately 160 nucleotides not shown in sequence; 5'Cap, Capl; fully modified with 5-methylcytidine and pseudouridine) G-CSF mRNA having an alpha-globin 3'UTR and a miR-122 binding site (G-CSF miR-122) (mRNA sequence is shown in SEQ ID NO: 6600; polyA tail of approximately 160 nucleotides not shown in sequence; 5'Cap, Capl; fully modified with 5-methylcytidine and pseudouridine) or G-CSF mRNA having an alpha-globin 3'UTR with four miR-122 binding sites with the seed deleted (G-CSF no seed) (mRNA sequence is shown in SEQ ID NO: 6601; polyA tail of approximately 160 nucleotides not shown in sequence; 5'Cap, Capl; fully modified with 5-methylcytidine and pseudouridine) was tested at a concentration of 250 ng per well in 24 well plates. The expression of G-CSF was measured by ELISA and the results are shown in Table 14.

Table 14. miR-122 Binding Sites

	HEK293A	Hepatocytes
	Protein Expression	Protein Expression

	(ng/mL)	(ng/mL)
G-CSF alpha	99.85	8.18
G-CSF miR-122	87.67	0
G-CSF no seed	200.2	8.05

[001003] Since HEK293 cells do not express miR-122 there was no down-regulation of G-CSF protein from the sequence containing miR-122. Whereas, the human hepatocytes express high levels of miR-122 and there was a drastic down-regulation of G-CSF protein objserved when the G-CSF sequence contained the miR-122 target sequence. Consequently, the mRNA functioned as an auxotrophic mRNA.

Example 20. MYC Inhibitor D Study in Cell Lines

[001004] Cell lines of liver and lung cancer, such as those described herein, are transfected with MYC inhibitor **D** modified mRNA in saline or formulated as described herein or in International Application No PCT/US2012/69610, herein incorporated by reference in its entirety. To evaluate the selectivity and/or the effects of therapy with MYC inhibitor **D** modified mRNA, normal hepatocytes are also transfected with the MYC inhibitor **D** modified mRNA.

Example 21. Formulation of Signal-Sensor Polynucleotides

[001005] Signal-sensor polynucleotides are formulated in lipid nanoparticles as described herein, known in the art, and/or as described in International Application No PCT/US2012/69610, herein incorporated by reference in its entirety. For tumor delivery, the lipid nanoparticle formulations are adapted for efficient delivery prior to *in vitro* or *in vivo* administration. For targeted delivery and/or to reduce toxicity the signal-senor polynucleotides include at least one miR binding site.

[001006] The lipid nanoparticle formulations are administered by methods known in the art or described herein (e.g., intraveneous, intramuscular and/or intranasal) to liver and lung cancer models (e.g., those described herein and subcutaneous human xenografts in mice, orthotopic human xenografts in mice and transgenic/genetically engineered mouse models).

Example 22. Delivery of Signal-Sensor Polynucleotides to Mammals

[001007] Signal-sensor polynucleotides are formulated for *in vivo* delivery in a lung and/or liver cancer model (e.g., those described herein). The signal-sensor polynucleotides are formulated in lipid nanoparticles as described herein, known in the

art and/or described in International Application No PCT/US2012/69610, herein incorporated by reference in its entirety.

[001008] The lung and/or liver cancer models are analyzed for protein expression, apoptosis, toxicity, efficacy through tumor volume, liver enzyme levels and effect on tumor tissue to evaluate the effect of administration of the formulated signal-sensor polynucleotides on the lung and/or liver cancer models. Assays are used to evaluate protein expression of the signal-sensor polynucleotides. Apoptosis, toxicity, efficacy through tumor volume, liver enzyme levels and tumor tissue are evaluate using common methods known in the art.

Example 23. Dose Response

[001009] Nanoparticle formulations of 98N12-5 (NPA-005) and DLin-KC2-DMA (NPA-003) were tested at varying concentrations to determine the MFI of FL4 or mCherry (mRNA sequence shown in SEQ ID NO: 6602; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and pseudouridine) over a range of doses. The formulations tested are outlined in Table 15. To determine the optimal concentration of nanoparticle formulations of 98N12-5, varying concentrations of formulated modified RNA (100 ng, 10 ng, 1.0 ng, 0.1 ng and 0.01 ng per well) were tested in a 24-well plate of HEK293.

[001010] Human embryonic kidney epithelial (HEK293) (LGC standards GmbH, Wesel, Germany) were seeded on 96-well plates (Greiner Bio-one GmbH, Frickenhausen, Germany) and plates were precoated with collagen typel . HEK293 were seeded at a density of 30,000 cells per well in 100 µï cell culture medium. The cell culture medium was DMEM, 10% FCS, adding 2mM L-Glutamine, 1 mM Sodiumpyruvate and lx non-essential amino acids (Biochrom AG, Berlin, Germany) and 1.2 mg/ml Sodiumbicarbonate (Sigma-Aldrich, Munich, Germany). Formulations containing mCherry mRNA were added in quadruplicates directly after seeding the cells and incubated.

[001011] Cells were harvested by transferring the culture media supernatants to a 96-well Pro-Bind U-bottom plate (Beckton Dickinson GmbH, Heidelberg, Germany). Cells were trypsinized with ½ volume Trypsin/EDTA (Biochrom AG, Berlin, Germany), pooled with respective supernatants and fixed by adding one volume PBS/2%FCS (both

Biochrom AG, Berlin, Germany)/0.5% formaldehyde (Merck, Darmstadt, Germany). Samples then were submitted to a flow cytometer measurement with a 532nm excitation laser and the 610/20 filter for PE-Texas Red in a LSRII cytometer (Beckton Dickinson GmbH, Heidelberg, Germany). The mean fluorescence intensity (MFI) of all events were analyzed and the results of the FL4 MFI of each dose are shown in Table 16. Likewise, to determine the optimal concentration of nanoparticle formulations of DLin-KC2-DMA, varying concentrations of formulated modified RNA (250 ng 100 ng, 10 ng, 1.0 ng, 0.1 ng and 0.01 ng per well) were tested in a 24-well plate of HEK293, and the results of the FL4 MFI of each dose are shown in Table 17. Nanoparticle formulations of DLin-KC2-DMA were also tested at varying concentrations of formulated modified RNA (250 ng, 100 ng and 30 ng per well) in a 24 well plate of HEK293, and the results of the FL4 MFI of each dose are shown in Table 18. A dose of 1 ng/well for 98N12-5 and a dose of 10 ng/well for DLin-KC2-DMA were found to resemble the FL4 MFI of the background.

[001012] To determine how close the concentrations resembled the background, we utilized a flow cytometer with optimized filter sets for detection of mCherry expression, and were able to obtain results with increased sensitivity relative to background levels. Doses of 25 ng/well, 0.25 ng/well, 0.025 ng/well and 0.0025 ng/well were analyzed for 98N12-5 (NPA-005) and DLin-KC2-DMA (NPA-003) to determine the MFI of mCherry. As shown in Table 19, the concentration of 0.025 ng/well and lesser concentrations are similar to the background MFI level of mCherry which is about 386.125.

Table 15. Formulations

Formulation #	**NPA-003**	**NPA-005**
Lipid	DLin-KC2-DMA	98N12-5
Lipid/RNA wt/wt	20	15
Mean size	114 nm PDI: 0.08	106 nm PDI: 0.12

Table 16. HEK293, NPA-005, 24-well, n=4

Formulation	FL4 MFI
Untreated control	0.246
NPA-005 100 ng	2.2175
NPA-005 10 ng	0.651
NPA-005 1.0 ng	0.28425
NPA-005 0.1 ng	0.27675
NPA-005 0.01 ng	0.2865

Table 17. HEK293, NPA-003, 24-well, n=4

Formulation	FL4 MFI
Untreated control	0.3225
NPA-003 250 ng	2.9575
NPA-003 100 ng	1.255
NPA-003 10 ng	0.40025
NPA-003 1 ng	0.33025
NPA-003 0.1 ng	0.34625
NPA-003 0.01 ng	0.3475

Table 18. HEK293, NPA-003, 24-well, n=4

Formulation	FL4 MFI
Untreated control	0.27425
NPA-003 250 ng	5.6075
NPA-003 100 ng	3.7825
NPA-003 30 ng	1.5525

Table 19. Concentration and MFI

	MFI mCherry	
Formulation	NPA-003	NPA-005
25 ng/well	11963.25	12256.75
0.25 ng/well	1349.75	2572.75
0.025 ng/well	459.50	534.75
0.0025 ng/well	310.75	471.75

Example 24. LNP Formulations

[001013] Formulations of DLin-DMA, DLin-K-DMA, DLin-KC2-DMA, 98N12-5, C12-200 and DLin-MC3-DMA were incubated at a concentration of 60 ng/well or 62.5 ng/well in a plate of HEK293 and 62.5 ng/well in a plate of HepG2 cells for 24 hours to determine the MFI of mCherry (mRNA sequence shown in SEQ ID NO: 6602; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and pseudouridine) for each formulation.

[001014] Human embryonic kidney epithelial (HEK293) and hepatocellular carcinoma epithelial (HepG2) cells (LGC standards GmbH, Wesel, Germany) were seeded on 96-well plates (Greiner Bio-one GmbH, Frickenhausen, Germany) and plates for HEK293 cells were precoated with collagen typel . HEK293 were seeded at a density of 30,000 and HepG2 were seeded at a density of 35,000 cells per well in 100 µï cell culture medium. For HEK293 the cell culture medium was DMEM, 10% FCS, adding 2mM L-

Glutamine, 1 mM Sodiumpyruvate and 1x non-essential amino acids (Biochrom AG, Berlin, Germany) and 1.2 mg/ml Sodiumbicarbonate (Sigma-Aldrich, Munich, Germany) and for HepG2 the culture medium was MEM (Gibco Life Technologies, Darmstadt, Germany), 10% FCS adding 2mM L-Glutamine, 1 mM Sodiumpyruvate and 1x non-essential amino acids (Biochrom AG, Berlin, Germany. Formulations containing mCherry mRNA (mRNA sequence shown in SEQ ID NO: 6602; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl); were added in quadruplicates directly after seeding the cells and incubated. The mCherry cDNA with the T7 promoter, 5'untranslated region (UTR) and 3' UTR used in *in vitro* transcription (IVT) is given in SEQ ID NO: 6603. The mCherry mRNA was modified with 5meC at each cytidine and pseudouridine replacement at each uridine site.

[001015] Cells were harvested by transferring the culture media supernatants to a 96-well Pro-Bind U-bottom plate (Beckton Dickinson GmbH, Heidelberg, Germany). Cells were trypsinized with ½ volume Trypsin/EDTA (Biochrom AG, Berlin, Germany), pooled with respective supernatants and fixed by adding one volume PBS/2%FCS (both Biochrom AG, Berlin, Germany)/0.5% formaldehyde (Merck, Darmstadt, Germany). Samples then were submitted to a flow cytometer measurement with a 532nm excitation laser and the 610/20 filter for PE-Texas Red in a LSRII cytometer (Beckton Dickinson GmbH, Heidelberg, Germany). The mean fluorescence intensity (MFI) of all events was determined.

[001016] The formulations tested are outlined in Table 20 below. As shown in Table 21 for the 60 ng/well and Tables 22, 23, 24 and 25 for the 62.5 ng/well,the formulation of NPA-003 and NPA-018 have the highest mCherry MFI and the formulations of NPA-008, NPA-010 and NPA-013 are most the similar to the background sample mCherry MFI value.

Table 20. Formulations

Formulation #	Lipid	Lipid/RNA wt/wt	Mean size (nm)
NPA-001	DLin-KC2-DMA	10	155 nm PDI: 0.08
NPA-002	DLin-KC2-DMA	15	140 nm PDI: 0.11
NPA-002-2	DLin-KC2-DMA	15	105 nm PDI: 0.04

NPA-003	DLin-KC2-DMA	20	114 nm PDI: 0.08
NPA-003-2	DLin-KC2-DMA	20	95 nm PDI: 0.02
NPA-005	98N12-5	15	127 nm PDI: 0.12
NPA-006	98N12-5	20	126 nm PDI: 0.08
NPA-007	DLin-DMA	15	148 nm PDI: 0.09
NPA-008	DLin-K-DMA	15	121 nm PDI: 0.08
NPA-009	C12-200	15	138 nm PDI: 0.15
NPA-010	DLin-MC3-DMA	15	126 nm PDI: 0.09
NPA-012	DLin-DMA	20	86 nm PDI: 0.08
NPA-013	DLin-K-DMA	20	104 nm PDI: 0.03
NPA-014	C12-200	20	101 nm PDI: 0.06
NPA-015	DLin-MC3-DMA	20	109 nm PDI: 0.07

Table 21. HEK293, 96-well, 60 ng Modified RNA/well

Formulation	MFI mCherry
Untreated	871.81
NPA-001	6407.25
NPA-002	14995
NPA-003	29499.5
NPA-005	3762
NPA-006	2676
NPA-007	9905.5
NPA-008	1648.75
NPA-009	2348.25
NPA-010	4426.75
NPA-012	11466
NPA-013	2098.25
NPA-014	3194.25
NPA-015	14524

Table 22. HEK293, 62.5 ng /well

Formulation	MFI mCherry
Untreated	871.81
NPA-001	6407.25
NPA-002	14995
NPA-003	29499.5

NPA-005	3762
NPA-006	2676
NPA-007	9905.5
NPA-008	1648.75
NPA-009	2348.25
NPA-010	4426.75
NPA-012	11466
NPA-013	2098.25
NPA-014	3194.25
NPA-015	14524

Table 23. HEK293, 62.5 ng /well

Formulation	MFI mCherry
Untreated	295
NPA-007	3504
NPA-012	8286
NPA-017	6128
NPA-003-2	17528
NPA-018	34142
NPA-010	1095
NPA-015	5859
NPA-019	3229

Table 24. HepG2, 62.5 ng /well

Formulation	MFI mCherry
Untreated	649.94
NPA-001	6006.25
NPA-002	8705
NPA-002-2	15860.25
NPA-003	15059.25
NPA-003-2	28881
NPA-005	1676
NPA-006	1473
NPA-007	15678
NPA-008	2976.25
NPA-009	961.75
NPA-010	3301.75
NPA-012	18333.25
NPA-013	5853
NPA-014	2257
NPA-015	16225.75

Table 25. HepG2, 62.5 ng /well

Formulation	MFI mCherry
Untreated control	656
NPA-007	16798

NPA-012	21993
NPA-017	20377
NPA-003-2	35651
NPA-018	40154
NPA-010	2496
NPA-015	19741
NPA-019	16373

Example 25. LNP *in vivo* studies

[001017] mCherry mRNA (SEQ ID NO: 6604; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytidine and pseudouridine) was formulated as a lipid nanoparticle (LNP) using the syringe pump method. The LNP was formulated at a 20: 1 weight ratio of total lipid to modified mRNA with a final lipid molar ratio of 50:10:38.5:1.5 (DLin-KC2-DMA: DSPC: Cholesterol: PEG-c-DOMG). The mCherry formulation, listed in Table 26, was characterized by particle size, zeta potential, and encapsulation.

Table 26. mCherry Formulation

Formulation #	**NPA-003-5**
Modified mRNA	mCherry
Mean size	105 nm PDI: 0.09
Zeta at pH 7.4	1.8 mV
Encaps. (RiboGr)	100%

[001018] The LNP formulation was administered to mice (n=5) intravenously at a modified mRNA dose of 100 ug. Mice were sacrificed at 24 hrs after dosing. The liver and spleen from the mice administered with mCherry modified mRNA formulations were analyzed by immunohistochemistry (IHC), western blot, or fluorescence-activated cell sorting (FACS).

[001019] Histology of the liver showed uniform mCherry expression throughout the section, while untreated animals did not express mCherry. Western blots were also used to confirm mCherry expression in the treated animals, whereas mCherry was not detected in the untreated animals. Tubulin was used as a control marker and was detected in both treated and untreated mice, indicating that normal protein expression in hepatocytes was unaffected.

[001020] FACS and IHC were also performed on the spleens of mCherry and untreated mice. All leukocyte cell populations were negative for mCherry expression by FACS analysis. By IHC, there were also no observable differences in the spleen in the spleen between mCherry treated and untreated mice.

Example 26. Titration of the binding affinity between two cofactors

[001021] Experiments are conducted in order to titrate the binding affinity between two cofactors. As used herein, the term "titrate" refers to a method whereby one or more factors are introduced systematically (such as at increasing levels or wherein the one or more factors are systematically modified) to a solution, scenario or series thereof in order to assess a property of interest. In this embodiment, the property of interest is the binding affinity between two cofactors. In one embodiment, constructs encoding the two cofactors are obtained and/or synthesized and a series of mutant constructs are prepared and/or synthesized. Mutant constructs encode cofactor mutants that may include truncated mutants (mutant proteins lacking one or more amino acids from either the N- or C-terminal domains), mutants with regional deletions [proteins wherein internal regions (comprising one or more amino acids) of the protein are absent], mutants with single amino acid substitutions (wherein a normally expressed amino acid is replaced with an alternative amino acid), mutants with one or more additional amino acids added internally or at either terminus, mutants with regional substitutions [proteins wherein internal regions (comprising one or more amino acids) of the protein are substituted with alternative regions (comprising one or more amino acids) and/or combinations of any of these. Mutant constructs are mutated randomly or subjected to targeted mutation based on existing knowledge of the molecular interactions necessary for binding between the two cofactors being investigated.

[001022] In some embodiments, a series of mutant proteins are designed such that the mutations follow a progressive pattern along the polypeptide chain. Such series may allow for a better understanding of a particular aspect or feature of the interaction between cofactors. A mutant series may include, for example, the production of a series of mutants, each with a single amino acid substitution, wherein each mutant has a different amino acid along it's polypeptide sequence mutated (e.g. alanine is substituted, thereby eliminating the influence of an amino acid side chain at each position). In another

example, a series of mutants are designed such that the mutants in the series comprise truncations of increasing size. In another example, amino acids capable of being post-translationally modified (e.g. phosphorylated, acetylated, ubiquitinated, glycosylated, etc.) in a similar manner may be mutated along the polypeptide sequence in a series of mutants.

[001023] For titration experiments with mutant cofactors, a baseline affinity between the two cofactors is established by combining both cofactors under conditions favorable for binding and the binding affinity between the cofactors is assayed. Binding affinity may be assessed using any of a variety of methods known in the art. Such methods may include, but are not limited to Western blot analysis, immunoprecipitation, enzyme-linked immunosorbent assay (ELISA), fluorescence resonance energy transfer (FRET), fluorescence recovery after photobleaching (FRAP), fluorescence polarization technologies and/or surface plasmon resonance (SPR) based technologies. For titration, according to one method, a mutant series of one or both cofactors are combined with the two unmutated cofactors (to allow for binding competition between the wild type and mutated proteins). Changes in affinity between the two cofactors in the presence of increasing concentrations of different mutants are assessed and compared and/or plotted against the specific mutations present in the series of mutants that are competing for binding. Alternatively, mutant cofactors in a series are individually combined with a corresponding unmutated binding partner and assessed for binding affinity. Increasing concentrations of the wild type cofactor (corresponding to the mutant cofactor) are introduced and changes in binding between the mutant cofactors and the corresponding unmutated binding partner are assessed. Comparisons are made between the resulting binding curves and the binding curves of other mutants tested.

[001024] In some embodiments, titration of the binding affinity between two cofactors is assessed in the presence or absence of increasing concentrations of a third factor. Such a third factor may be an inhibitor or activator of binding between the two cofactors. A series of mutants, as described above, may be generated for a third factor and such a series may be used in titration experiments to assess the effect of mutations on binding between the two cofactors.

[001025] Information obtained from titration experiments may be used to design modified mRNA molecules to encode factors that modulate the interaction between cofactors.

[001026] In some embodiments, titration experiments are carried out wherein the binding affinity between HIFl subunits (HIFl - alpha, HIF2-alpha and ARNT) and/or mutated HIFl subunits and/or other proteins that interact with HIFl is assessed. Titration experiments may utilize mutant series generated using constructs for one or more of HIFl -alpha, HIF2-alpha, ARNT and/or a third interacting factor. In some embodiments, a mutant series is generated for HIFl -alpha. HIFl -alpha and HIF2-alpha are hyrdroxylated by HIF hydroxylase enzymes under normal levels of oxygen in the cell, facilitating degredation and/or blocking transcriptional activity. Hyrdorxylation decreases as oxygen levels drop, allowing HIFl -alpha and/or HIF2-alpha to associate with their cofactor, ARNT leading to elevated expression of genes comprising HIF-response elements (HREs) (Keith, B. et al., HIF l a and HIF2a: sibling rivalry in hypoxic tumour growth and progression. Nat Rev Cancer. 201 1 Dec 15;12(l):9-22). In one embodiment, HIFl-alpha mutant series are generated wherein mutations in the series progressively eliminate one or more hydroxylation sites along the polypeptide chain (including, but not limited to proline 402, proline 564 and/or asparagine 803), thereby modulating stability and/or transcriptional activity in mutant versions of HIFl-alpha. In another embodiment, an alternative cofactor, HIF2-alpha is used to generate a mutant series. Such a mutant series may progressively eliminate one or more hydroxylation sites along the polypeptide chain (including, but not limited to proline 405, proline 531 and/or asparagine 847), thereby modulating stability and/or transcriptional activity in mutant versions of HIF2-alpha. In another embodiment, HIFl-alpha and/or HIF2-alpha mutant series are generated that progressively mutate regions necessary for interaction with ARNT, thereby creating mutants with altered abilities to bind ARNT and modulate HIF-dependent gene expression. In another embodiment, ARNT mutant series are generated that progressively mutate regions necessary for interactions with other HIF subunits, thereby creating mutants with altered abilities to bind HIF subunits and modulate HIF-dependent gene expression.

[001027] In some embodiments, mutant series are generated for Von Hippel-Landau tumor suppressor protein (pVHL). This protein binds hydroxylated HIFl -alpha and HIF2-alpha, facilitating their ubiquitination and degradation. In one embodiment, mutant series are generated that progressively mutate regions necessary for interaction with HIFl subunits, thereby creating mutants with altered abilities to bind HIFl subunits and modulate HIF-dependent gene expression.

[001028] Shown in Table 27 and 28 are the transcript sequences and polypeptide sequences (respectively) for protein targets for use in titration experiments. The name and description of the gene encoding the polypeptide of interest are accompanied by the ENSEMBL Transcript ID (ENST) and transcript sequence (Table 27) or the ENSEMBL Protein ID (ENSP) and peptide sequence (Table 28). In some embodiments of the present invention, modified mRNAs may be designed to encode factors that modulate the affinity between HIF subunits and/or HIF-dependent gene expression. Such modified mRNAs may be designed using knowledge gained from titration experiments.

Table 27. Transcript sequences for additional targets for titration experiments

Target	Target Description	ENST ID	Transcript Sequence	SEQ ID NO
HIF2-alpha	hypoxia inducible factor 2, alpha subunit; endothelial PAS domain protein **1**	263734	GCTTTACACTCGCGAGCGGACCGCCACACGG GTCCGGTGCCCGCTGCGCTTCCGCCCCAGCGC TCCTGAGGCGGCCGTACAATCCTCGGCAGTGT CCTGAGACTGTATGGTCAGCTCAGCCCGGCCT CCGACTCCTTCCGACTCCCAGCATTCGAGCCA CTTTTTTTTTTCTTTGAAAACTCAGAAAAGTG ACTCCTTTTCCAGGGAAAAAGGAACTTGGGTT CCCTTCTCTCCGTCCTCTTTTCGGGTCTGACAG CCTCCACCCACTCCTTCCCCGGACCCCGCCTC CGCGCGCAGGTTCCTCCCAGTCACCTTTCTCC ACCCCCGCCCCCGCACCTAGCCCGCCGCGCG CCACCTTCCACCTGACTGCGCGGGGCGCTCGG GACCTGCGCGCACCTCGGACCTTCACCACCCG CCCGGGCCGCGGGGAGCGGACGAGGGCCACA GCCCCCCACCCGCCAGGGAGCCCAGGTGCTC GGCGTCTGAACGTCTCAAAGGGCCACAGCGA CAATGACAGCTGACAAGGAGAAGAAAAGGA GTAGCTCGGAGAGGAGGAAGGAGAAGTCCCG GGATGCTGCGCGGTGCCGGCGGAGCAAGGAG ACGGAGGTGTTCTATGAGCTGGCCCATGAGC TGCCTCTGCCCCACAGTGTGAGCTCCCATCTG GACAAGGCCTCCATCATGCGACTGGCAATCA GCTTCCTGCGAACACACAAGCTCCTCTCCTCA	6605

			GTTTGCTCTGAAAACGAGTCCGAAGCCGAAG CTGACCAGCAGATGGACAACTTGTACCTGAA AGCCTTGGAGGGTTTCATTGCCGTGGTGACCC AAGATGGCGACATGATCTTTCTGTCAGAAAA CATCAGCAAGTTCATGGGACTTACACAGGTG GAGCTAACAGGACATAGTATCTTTGACTTCAC TCATCCCTGCGACCATGAGGAGATTCGTGAG AACCTGAGTCTCAAAAATGGCTCTGGTTTTGG GAAAAAAAGCAAAGACATGTCCACAGAGCG GGACTTCTTCATGAGGATGAAGTGCACGGTC ACCAACAGAGGCCGTACTGTCAACCTCAAGT CAGCCACCTGGAAGGTCTTGCACTGCACGGG CCAGGTGAAAGTCTACAACAACTGCCCTCCTC ACAATAGTCTGTGTGGCTACAAGGAGCCCCT GCTGTCCTGCCTCATCATCATGTGTGAACCAA TCCAGCACCCATCCCACATGGACATCCCCCTG GATAGCAAGACCTTCCTGAGCCGCCACAGCA TGGACATGAAGTTCACCTACTGTGATGACAG AATCACAGAACTGATTGGTTACCACCCTGAG GAGCTGCTTGGCCGCTCAGCCTATGAATTCTA CCATGCGCTAGACTCCGAGAACATGACCAAG AGTCACCAGAACTTGTGCACCAAGGGTCAGG TAGTAAGTGGCCAGTACCGGATGCTCGCAAA GCATGGGGGCTACGTGTGGCTGGAGACCCAG GGGACGGTCATCTACAACCCTCGCAACCTGC AGCCCCAGTGCATCATGTGTGTCAACTACGTC CTGAGTGAGATTGAGAAGAATGACGTGGTGT TCTCCATGGACCAGACTGAATCCCTGTTCAAG CCCCACCTGATGGCCATGAACAGCATCTTTGA TAGCAGTGGCAAGGGGGCTGTGTCTGAGAAG AGTAACTTCCTATTCACCAAGCTAAAGGAGG AGCCCGAGGAGCTGGCCCAGCTGGCTCCCAC CCCAGGAGACGCCATCATCTCTCTGGATTTCG GGAATCAGAACTTCGAGGAGTCCTCAGCCTA TGGCAAGGCCATCCTGCCCCCGAGCCAGCCA TGGGCCACGGAGTTGAGGAGCCACAGCACCC AGAGCGAGGCTGGGAGCCTGCCTGCCTTCAC CGTGCCCCAGGCAGCTGCCCCGGGCAGCACC ACCCCCAGTGCCACCAGCAGCAGCAGCAGCT GCTCCACGCCCAATAGCCCTGAAGACTATTAC ACATCTTTGGATAACGACCTGAAGATTGAAG TGATTGAGAAGCTCTTCGCCATGGACACAGA GGCCAAGGACCAATGCAGTACCCAGACGGAT TTCAATGAGCTGGACTTGGAGACACTGGCAC CCTATATCCCCATGGACGGGGAAGACTTCCA GCTAAGCCCCATCTGCCCCGAGGAGCGGCTC TTGGCGGAGAACCCACAGTCCACCCCCCAGC ACTGCTTCAGTGCCATGACAAACATCTTCCAG CCACTGGCCCCTGTAGCCCCGCACAGTCCCTT CCTCCTGGACAAGTTTCAGCAGCAGCTGGAG AGCAAGAAGACAGAGCCCGAGCACCGGCCCA	

			TGTCCTCCATCTTCTTTGATGCCGGAAGCAAA GCATCCCTGCCACCGTGCTGTGGCCAGGCCA GCACCCCTCTCTCTTCCATGGGGGGCAGATCC AATACCCAGTGGCCCCCAGATCCACCATTAC ATTTTGGGCCCACAAAGTGGGCCGTCGGGGA TCAGCGCACAGAGTTCTTGGGAGCAGCGCCG TTGGGGCCCCCTGTCTCTCCACCCCATGTCTC CACCTTCAAGACAAGGTCTGCAAAGGGTTTT GGGGCTCGAGGCCCAGACGTGCTGAGTCCGG CCATGGTAGCCCTCTCCAACAAGCTGAAGCT GAAGCGACAGCTGGAGTATGAAGAGCAAGCC TTCCAGGACCTGAGCGGGGGGGACCCACCTG GTGGCAGCACCTCACATTTGATGTGGAAACG GATGAAGAACCTCAGGGGTGGGAGCTGCCCT TTGATGCCGGACAAGCCACTGAGCGCAAATG TACCCAATGATAAGTTCACCCAAAACCCCAT GAGGGGCCTGGGCCATCCCCTGAGACATCTG CCGCTGCCACAGCCTCCATCTGCCATCAGTCC CGGGGAGAACAGCAAGAGCAGGTTCCCCCCA CAGTGCTACGCCACCCAGTACCAGGACTACA GCCTGTCGTCAGCCCACAAGGTGTCAGGCAT GGCAAGCCGGCTGCTCGGGCCCTCATTTGAGT CCTACCTGCTGCCCGAACTGACCAGATATGAC TGTGAGGTGAACGTGCCCGTGCTGGGAAGCT CCACGCTCCTGCAAGGAGGGGACCTCCTCAG AGCCCTGGACCAGGCCACCTGAGCCAGGCCT TCTACCTGGGCAGCACCTCTGCCGACGCCGTC CCACCAGCTTCACTCTCTCCGTCTGTTTTTGCA ACTAGGTATTTCTAACGCCAGCACACTATTTA CAAGATGGACTTACCTGGCAGACTTGCCCAG GTCACCAAGCAGTGGCCTTTTTCTGAGATGCT CACTTTATTATCCCTATTTTTAAAGTACACAA TTGTTTTACCTGTTCTGAAATGTTCTTAAATTT TGTAGGATTTTTTTCCTCCCCACCTTCAATGA CTTCTAATTTATATTATCCATAGGTTTCTCTCC CTCCTTCTCCTTCTCACACACAACTGTCCATA CTAACAAGTTTGGTGCATGTCTGTTCTTCTGT AGGGAGAAGCTTTAGCTTCATTTTACTAAAAA GATTCCTCGTTATTGTTGTTGCCAAAGAGAAA CAAAAATGATTTTGCTTTCCAAGCTTGGTTTG TGGCGTCTCCCTCGCAGAGCCCTTCTCGTTTC TTTTTTAAACTAATCACCATATTGTAAATTTC AGGGTTTTTTTTTTTTTGTTTAAGCTGACTCTT TGCTCTAATTTTGGAAAAAAAGAAATGTGAA GGGTCAACTCCAACGTATGTGGTTATCTGTGA AAGTTGCACAGCGTGGCTTTTCCTAAACTGGT GTTTTTCCCCCGCATTTGGTGGATTTTTTATTA TTATTCAAAAACATAACTGAGTTTTTTAAAAG AGGAGAAAATTTATATCTGGGTTAAGTGTTTA TCATATATATGGGTACTTTGTAATATCTAAAA ACTTAGAAACGGAAATGGAATCCTGCTCACA	

			AAATCACTTTAAGATCTTTTCGAAGCTGTTAA TTTTTCTTAGTGTTGTGGACACTGCAGACTTG TCCAGTGCTCCCACGGCCTGTACGGACACTGT GGAAGGCCTCCCTCTGTCGGCTTTTTGCCATC TGTGATATGCCATAGGTGTGACAATCCGAGC AGTGGAGTCATTCAGCGGGAGCACTGCGCGC TATCCCCTCACATTCTCTATGTACTATGTATGT ATGTATTATTATTATTGCTGCCAAGAGGGTCT GATGGCACGTTGTGGGGTCGGGGGGTGGGGC GGGGAAGTGCTCTAACTTTTCTTAAGGTTTTG TTGCTAGCCCTTCAAGTGCACTGAGCTATGTG ACTCGGATGGTCTTTCACACGGCACATTTGGA CATTTCCAGAACTACCATGAGATGGTTTAGAC GGGAATTCATGCAAATGAGGGGTCAAAAATG GTATAGTGACCCCGTCCACGTCCTCCAAGCTC ACGACCTTGGAGCCCCGTGGAGCTGGACTGA GGAGGAGGCTGCACAGCGGGAGAGCAGCTG GTCCAGACCAGCCCTGCAGCCCCCACTCAGC CGGCAGCCAGATGGCCCCGCAAGGCCTCCAG GGATGGCCCCTAGCCACAGGCCCTGGCTGAG GTCTCTGGGTCGGTCAGTGACATGTAGGTAG GAAGCACTGAAAATAGTGTTCCCAGAGCACT TTGCAACTCCCTGGGTAAGAGGGACGACACC TCTGGTTTTTCAATACCAATTACATGGAACTT TTCTGTAATGGGTACAATGAAGAAGTTTCTAA AAACACACACAAAGCACATTGGGCCAACTAT TTAGTAAGCCCGGATAGACTTATTGCCAAAA ACAAAAAATAGCTTTCAAAAGAAATTTAAGT TCTATGAGAAATTCCTTAGTCATGGTGTTGCG TAAATCATATTTTAGCTGCACGGCATTACCCC ACACAGGGTGGCAGAACTTGAAGGGTTACTG ACGTGTAAATGCTGGTATTTGATTTCCTGTGT GTGTTGCCCTGGCATTAAGGGCATTTTACCCT TGCAGTTTTACTAAAACACTGAAAAATATTCC AAGCTTCATATTAACCCTACCTGTCAACGTAA CGATTTCATGAACGTTATTATATTGTCGAATT CCTACTGACAACATTATAACTGTATGGGAGCT TAACTTTATAAGGAAATGTATTTTGACACTGG TATCTTATTAAAGTATTCTGATCCTA	
pVHL	von Hippel-Lindau tumor suppressor	256474	TGAGTGTTTATGTTTGTAGTTTTAATTGCTCTG AAGTAAATATCTGATTTTCCAATTTCCACCAG AGTGCTCTGCACATAGTAGGTCTAATTATTTT TCCCTCTTTACTAATCACCCATGCCTTGTAAG AATTCAGTTAGTTGACTTTTTGTACTTTATAA GCGTGATGATTGGGTGTTCCCGTGTGAGATGC GCCACCCTCGAACCTTGTTACGACGTCGGCAC ATTGCGCGTCTGACATGAAGAAAAAAAAAAT TCAGTTAGTCCACCAGGCACAGTGGCTAAGG CCTGTAATCCCTGCACTTTGAGAGGCCAAGGC AGGAGGATCACTTGAACCCAGGAGTTCGAGA	6606

			CCAGCCTAGGCAACATAGCGAGACTCCGTTT CAAACAACAAATAAAAATAATTAGTCGGGCA TGGTGGTGCGCGCCTACAGTACCAACTACTCG GGAGGCTGAGGCGAGACGATCGCTTGAGCCA GGGAGGTCAAGGCTGCAGTGAGCCAAGCTCG CGCCACTGCACTCCAGCCCGGGCGACAGAGT GAGACCCTGTCTCAAAAAAAAAAAAAACACC AAACCTTAGAGGGGCGAAAAAAAATTTTATA GTGGAAATACAGTAACGAGTTGGCCTAGCCT CGCCTCCGTTACAACGGCCTACGGTGCTGGA GGATCCTTCTGCGCACGCGCACAGCCTCCGGC CGGCTATTTCCGCGAGCGCGTTCCATCCTCTA CCGAGCGCGCGCGAAGACTACGGAGGTCGAC TCGGGAGCGCGCACGCAGCTCCGCCCCGCGT CCGACCCGCGGATCCCGCGGCGTCCGGCCCG GGTGGTCTGGATCGCGGAGGGAATGCCCCGG AGGGCGGAGAACTGGGACGAGGCCGAGGTA GGCGCGGAGGAGGCAGGCGTCGAAGAGTAC GGCCCTGAAGAAGACGGCGGGGAGGAGTCG GGCGCCGAGGAGTCCGGCCCGGAAGAGTCCG GCCCGGAGGAACTGGGCGCCGAGGAGGAGAT GGAGGCCGGGCGGCCGCGGCCCGTGCTGCGC TCGGTGAACTCGCGCGAGCCCTCCCAGGTCAT CTTCTGCAATCGCAGTCCGCGCGTCGTGCTGC CCGTATGGCTCAACTTCGACGGCGAGCCGCA GCCCTACCCAACGCTGCCGCCTGGCACGGGC CGCCGCATCCACAGCTACCGAGGTCACCTTTG GCTCTTCAGAGATGCAGGGACACACGATGGG CTTCTGGTTAACCAAACTGAATTATTTGTGCC ATCTCTCAATGTTGACGGACAGCCTATTTTTG CCAATATCACACTGCCAGTGTATACTCTGAAA GAGCGATGCCTCCAGGTTGTCCGGAGCCTAG TCAAGCCTGAGAATTACAGGAGACTGGACAT CGTCAGGTCGCTCTACGAAGATCTGGAAGAC CACCCAAATGTGCAGAAAGACCTGGAGCGGC TGACACAGGAGCGCATTGCACATCAACGGAT GGGAGATTGAAGATTTCTGTTGAAACTTACAC TGTTTCATCTCAGCTTTTGATGGTACTGATGA GTCTTGATCTAGATACAGGACTGGTTCCTTCC TTAGTTTCAAAGTGTCTCATTCTCAGAGTAAA ATAGGCACCATTGCTTAAAAGAAAGTTAACT GACTTCACTAGGCATTGTGATGTTTAGGGGCA AACATCACAAAATGTAATTTAATGCCTGCCCA TTAGAGAAGTATTTATCAGGAGAAGGTGGTG GCATTTTTGCTTCCTAGTAAGTCAGGACAGCT TGTATGTAAGGAGGTTTGTATAAGTAATTCAG TGGGAATTGCAGCATATCGTTTAATTTTAAGA AGGCATTGGCATCTGCTTTTAATGGATGTATA ATACATCCATTCTACATCCGTAGCGGTTGGTG ACTTGTCTGCCTCCTGCTTTGGGAAGACTGAG GCATCCGTGAGGCAGGGACAAGTCTTTCTCCT	

			CTTTGAGACCCCAGTGCCTGCACATCATGAGC CTTCAGTCAGGGTTTGTCAGAGGAACAAACC AGGGGACACTTTGTTAGAAAGTGCTTAGAGG TTCTGCCTCTATTTTTGTTGGGGGGTGGGAGA GGGGACCTTAAAATGTGTACAGTGAACAAAT GTCTTAAAGGGAATCATTTTTGTAGGAAGCAT TTTTTATAATTTTCTAAGTCGTGCACTTTCTCG GTCCACTCTTGTTGAAGTGCTGTTTTATTACT GTTTCTAAACTAGGATTGACATTCTACAGTTG TGATAATAGCATTTTTGTAACTTGCCATCCGC ACAGAAAATACGAGAAAATCTGCATGTTTGA TTATAGTATTAATGGACAAATAAGTTTTTGCT AAATGTGAGTATTTCTGTTCCTTTTTGTAAAT ATGTGACATTCCTGATTGATTTGGGTTTTTTTG TTGTTGTTGTTTTGTTTTGTTTTGTTTTTTTGAG ATGGAGTCTCACTCTTGTCACCCAGGCTGGAG TGCAGTGGCGCCATCTCGGCTCACTGCAACCT CTGCCTCCTGGGTTCACGTAATCCTCCTGAGT AGCTGGGATTACAGGCGCCTGCCACCACGCT GGCCAATTTTTGTACTTTTAGTAGAGACAGTG TTTCGTCATGTTGGCCAGGCTGGTTTCAAACT CCTGACCTCAGGTGATCCGCCCACCTCAGCCT CCCAAAATGGTGGGATTACAGGTGTGTGGGC CACCGTGCCTGGCTGATTCAGCATTTTTTATC AGGCAGGACCAGGTGGCACTTCCACCTCCAG CCTCTGGTCCTACCAATGGATTCATGGAGTAG CCTGGACTGTTTCATAGTTTTCTAAATGTACA AATTCTTATAGGCTAGACTTAGATTCATTAAC TCAAATTCAATGCTTCTATCAGACTCAGTTTT TTGTAACTAATAGATTTTTTTTTCCACTTTTGT TCTACTCCTTCCCTAATAGCTTTTTAAAAAAA TCTCCCCAGTAGAGAAACATTTGGAAAAGAC AGAAAACTAAAAAGGAAGAAAAAAGATCCC TATTAGATACACTTCTTAAATACAATCACATT AACATTTTGAGCTATTTCCTTCCAGCCTTTTTA GGGCAGATTTTGGTTGGTTTTTACATAGTTGA GATTGTACTGTTCATACAGTTTTATACCCTTTT TCATTTAACTTTATAACTTAAATATTGCTCTAT GTTAGTATAAGCTTTTCACAAACATTAGTATA GTCTCCCTTTTATAATTAATGTTTGTGGGTATT TCTTGGCATGCATCTTTAATTCCTTATCCTAGC CTTTGGGCACAATTCCTGTGCTCAAAAATGAG AGTGACGGCTGGCATGGTGGCTCCCGCCTGT AATCCCAGTACTTTGGAAAGCCAAGGTAAGA GGATTGCTTGAGCCCAGAACTTCAAGATGAG CCTGGGCTCATAGTGAGAACCCATCTATACA AAAAATTTTTAAAAATTAGCATGGCGGCACA CATCTGTAATCCTAGCTACTTGGCAGGCTGAG GTGAGAAGATCATTGGAGTTTAGGAATTGGA GGCTGCAGTGAGCCATGAGTATGCCACTGCA CTCCAGCCTGGGGGACAGAGCAAGACCCTGC	

			CTCAAAAAAAAAAAAAAAAAAAAATCAGG CCGGGCATGGTGGCTCACGCCTGTAATCCCA GCACTTTGGGAGGTCGAGGTGGGCAGATCAC CTGAGGTCAGGAGTTCGAGACCAGCCTGGCC AACATGGTAAAACCCCATTTCTACTAAAAAA TACAAGAAT	
pVHL	von Hippel-Lindau tumor suppressor	345392	CCCGCGTCCGACCCGCGGATCCCGCGGCGTC CGGCCCGGGTGGTCTGGATCGCGGAGGGAAT GCCCCGGAGGGCGGAGAACTGGGACGAGGCC GAGGTAGGCGCGGAGGAGGCAGGCGTCGAA GAGTACGGCCCTGAAGAAGACGGCGGGGAG GAGTCGGGCGCCGAGGAGTCCGGCCCGGAAG AGTCCGGCCCGGAGGAACTGGGCGCCGAGGA GGAGATGGAGGCCGGGCGGCCGCGGCCCGTG CTGCGCTCGGTGAACTCGCGCGAGCCCTCCCA GGTCATCTTCTGCAATCGCAGTCCGCGCGTCG TGCTGCCCGTATGGCTCAACTTCGACGGCGAG CCGCAGCCCTACCCAACGCTGCCGCCTGGCA CGGGCCGCCGCATCCACAGCTACCGAGTGTA TACTCTGAAAGAGCGATGCCTCCAGGTTGTCC GGAGCCTAGTCAAGCCTGAGAATTACAGGAG ACTGGACATCGTCAGGTCGCTCTACGAAGAT CTGGAAGACCACCCAAATGTGCAGAAAGACC TGGAGCGGCTGACACAGGAGCGCATTGCACA TCAACGGATGGGAGATTGAAGATTTCTGTTG AAACTTACACTGTTTCATCTCAGCTTTTGATG GTACTGATGAGTCTTGATCTAGATACAGGACT GGTTCCTTCCTTAGTTTCAAAGTGTCTCATTCT CAGAGTAAAATAGGCACCATTGCTTAAAAGA AAGTTAACTGACTTCACTAGGCATTGTGATGT TTAGGGGCAAACATCACAAAATGTAATTTAA TGCCTGCCCATTAGAGAAGTATTTATCAGGAG AAGGTGGTGGCATTTTTGCTTCCTAGTAAGTC AGGACAGCTTGTATGTAAGGAGGTTTGTATA AGTAATTCAGTGGGAATTGCAGCATATCGTTT AATTTTAAGAAGGCATTGGCATCTGCTTTTAA TGGATGTATAATACATCCATTCTACATCCGTA GCGGTTGGTGACTTGTCTGCCTCCTGCTTTGG GAAGACTGAGGCATCCGTGAGGCAGGGACAA GTCTTTCTCCTCTTTGAGACCCCAGTGCCTGC ACATCATGAGCCTTCAGTCAGGGTTTGTCAGA GGAACAAACCAGGGGACACTTTGTTAGAAAG TGCTTAGAGGTTCTGCCTCTATTTTTGTTGGG GGGTGGGAGAGGGGACCTTAAAATGTGTACA GTGAACAAATGTCTTAAAGGGAATCATTTTTG TAGGAAGCATTTTTTATAATTTTCTAAGTCGT GCACTTTCTCGGTCCACTCTTGTTGAAGTGCT GTTTTATTACTGTTTCTAAACTAGGATTGACA TTCTACAGTTGTGATAATAGCATTTTTGTAAC TTGCCATCCGCACAGAAAATACGAGAAAATC	6607

			TGCATGTTTGATTATAGTATTAATGGACAAAT AAGTTTTTGCTAAATGTGAGTATTTCTGTTCC TTTTTGTAAATATGTGACATTCCTGATTGATTT GGGTTTTTTTGTTGTTGTTGTTTTGTTTTGTTTT GTTTTTTTGAGATGGAGTCTCACTCTTGTCAC CCAGGCTGGAGTGCAGTGGCGCCATCTCGGC TCACTGCAACCTCTGCCTCCTGGGTTCACGTA ATCCTCCTGAGTAGCTGGGATTACAGGCGCCT GCCACCACGCTGGCCAATTTTTGTACTTTTAG TAGAGACAGTGTTTCGTCATGTTGGCCAGGCT GGTTTCAAACTCCTGACCTCAGGTGATCCGCC CACCTCAGCCTCCCAAAATGGTGGGATTACA GGTGTGTGGGCCACCGTGCCTGGCTGATTCAG CATTTTTTATCAGGCAGGACCAGGTGGCACTT CCACCTCCAGCCTCTGGTCCTACCAATGGATT CATGGAGTAGCCTGGACTGTTTCATAGTTTTC TAAATGTACAAATTCTTATAGGCTAGACTTAG ATTCATTAACTCAAATTCAATGCTTCTATCAG ACTCAGTTTTTTGTAACTAATAGATTTTTTTTT CCACTTTTGTTCTACTCCTTCCCTAATAGCTTT TTAAAAAAATCTCCCCAGTAGAGAAACATTT GGAAAAGACAGAAAACTAAAAAGGAAGAAA AAAGATCCCTATTAGATACACTTCTTAAATAC AATCACATTAACATTTTGAGCTATTTCCTTCC AGCCTTTTTAGGGCAGATTTTGGTTGGTTTTT ACATAGTTGAGATTGTACTGTTCATACAGTTT TATACCCTTTTTCATTTAACTTTATAACTTAAA TATTGCTCTATGTTAGTATAAGCTTTTCACAA ACATTAGTATAGTCTCCCTTTTATAATTAATG TTTGTGGGTATTTCTTGGCATGCATCTTTAATT CCTTATCCTAGCCTTTGGGCACAATTCCTGTG CTCAAAAATGAGAGTGACGGCTGGCATGGTG GCTCCCGCCTGTAATCCCAGTACTTTGGAAAG CCAAGGTAAGAGGATTGCTTGAGCCCAGAAC TTCAAGATGAGCCTGGGCTCATAGTGAGAAC CCATCTATACAAAAAATTTTTAAAAATTAGCA TGGCGGCACACATCTGTAATCCTAGCTACTTG GCAGGCTGAGGTGAGAAGATCATTGGAGTTT AGGAATTGGAGGCTGCAGTGAGCCATGAGTA TGCCACTGCACTCCAGCCTGGGGGACAGAGC AAGACCCTGCCTCAAAAAAAAAAAAAAAAA AAAAA	
pVHL	von Hippel-Lindau tumor suppressor	450183	GGATCCCGCGGCGTCCGGCCCGGGTGGTCTG GATCGCGGAGGGAATGCCCCGGAGGGCGGAG AACTGGGACGAGGCCGAGGTAGGCGCGGAG GAGGCAGGCGTCGAAGAGTACGGCCCTGAAG AAGACAGCTACCGAGGTCACCTTTGGCTCTTC AGAGATGCAGGGACACACGATGGGCTTCTGG TTAACCAAACTGAATTATTTGTGCCATCTCTC AATGTTGACGGACAGCCTATTTTTGCCAATAT	6608

			CACACTGCCAGTGTATACTCTGAAAGAGCGA TGCCTCCAGGTTGTCCGGAGCCTAGTCAAGCC TGAGAATTACAGGAGACTGGACATCGTCAGG TCGCTCTACGAAGATCTGGAAGACCACCCAA ATGTGCAGAAAGACCTGGAGCGGCTGACACA GGAGCGCATTGCACATCAACGGATGGGAGAT TGAAGATTTCTGTTGAAACTTACACTGTTTCA TCTCAGCTTTTGATGGTACTGATGAGTCTTGA TCTAGATACAGGACTGGTTCCTTCCTTAGTTT CAAAGTGTCTCATTCTCAGAGTAAAATAGGC ACCATTGCTTAAAAGAAAGTTAACTGACTTCA CTAGGCATTGTGATGTTTAGGGGCAAACATC ACAAAATGTAATTTAATGCCTGCCCATTAGAG AAGTATTTATCAGGAGAAGGTGGTGGCATTTT TGCTTCCTAGTAAGTCAGGACAGCTTGTATGT AAGGAGGTTTGTATAAGTAATTCAGTGGGAA TTGCAGCATATCGTTTAATTTTAAGAAGGCAT TGGCATCTGCTTTTAATGGATGTATAATACAT CCATTCTACATCCGTAGCGGTTGGTGACTTGT CTGCCTCCTGCTTTGGGAAGACTGAGGCATCC GTGAGGCAGGGACAAGTCTTTCTCCTCTTTGA GACCCCAGTGCCTGCACATCATGAGCCTTCAG TCAGGGTTTGTCAGAGGAACAAACCAGGGGA CACTTTGTTAGAAAGTGCTTAGAGGTTCTGCC TCTATTTTTGTTGGGGGGTGGGAGAGGGGAC CTTAAAATGTGTACAGTGAACAAATGTCTTAA AGGGAATCATTTTTGTAGGAAGCATTTTTTAT AATTTTCTAAGTCGTGCACTTTCTCGGTCCAC TCTTGTT	
HIF1 -alpha	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	557538	ATTTGAAAACTTGGCAACCTTGGATTGGATGG ATTCATATTTCTTAGTATAGAAGTTCTTGATA TAACTGAAAAATTAAGTTAAACACTTAATAA GTGGTGGTTACTCAGCACTTTTAGATGCTGTT TATAATAGATGACCTTTTCTAACTAATTTACA GTTTTTTGAAAGATAACTGAGAGGTTGAGGG ACGGAGATTTTCTTCAAGCAATTTTTTTTTTCA TTTTAAATGAGCTCCCAATGTCGGAGTTTGGA AAACAAATTTGTCTTTTTAAAAGAAGGTCTAG GAAACTCAAAACCTGAAGAATTGGAAGAAAT CAGAATAGAAAATGGTAGGATAAGTTCTGAA CGTCGAAAAGAAAAGTCTCGAGATGCAGCCA GATCTCGGCGAAGTAAAGAATCTGAAGTTTTT TATGAGCTTGCTCATCAGTTGCCACTTCCACA TAATGTGAGTTCGCATCTTGATAAGGCCTCTG TGATGAGGCTTACCATCAGCTATTTGCGTGTG AGGAAACTTCTGGATGCTGGTGATTTGGATAT TGAAGATGACATGAAAGCACAGATGAATTGC TTTTATTTGAAAGCCTTGGATGGTTTTGTTAT GGTTCTCACAGATGATGGTGACATGATTTACA TTTCTGATAATGTGAACAAATACATGGGATTA	6609

			ACTCAGTTTGAACTAACTGGACACAGTGTGTT TGATTTTACTCATCCATGTGACCATGAGGAAA TGAGAGAAATGCTTACACACAGAAATGGCCT TGTGAAAAAGGGTAAAGAACAAAACACACA GCGAAGCTTTTTTCTCAGAATGAAGTGTACCC TAACTAGCCGAGGAAGAACTATGAACATAAA GTCTGCAACATGGAAGGTATTGCACTGCACA GGCCACATTCACGTATATGATACCAACAGTA ACCAACCTCAGTGTGGGTATAAGAAACCACC TATGACCTGCTTGGTGCTGATTTGTGAACCCA TTCCTCACCCATCAAATATTGAAATTCCTTTA GATAGCAAGACTTTCCTCAGTCGACACAGCCT GGATATGAAATTTTCTTATTGTGATGAAAGAA TTACCGAATTGATGGGATATGAGCCAGAAGA ACTTTTAGGCCGCTCAATTTATGAATATTATC ATGCTTTGGACTCTGATCATCTGACCAAAACT CATCATGATATGTTTACTAAAGGACAAGTCAC CACAGGACAGTACAGGATGCTTGCCAAAAGA GGTGGATATGTCTGGGTTGAAACTCAAGCAA CTGTCATATATAACACCAAGAATTCTCAACCA CAGTGCATTGTATGTGTGAATTACGTTGTGAG TGGTATTATTCAGCACGACTTGATTTTCTCCC TTCAACAAACAGAATGTGTCCTTAAACCGGTT GAATCTTCAGATATGAAAATGACTCAGCTATT CACCAAAGTTGAATCAGAAGATACAAGTAGC CTCTTTGACAAACTTAAGAAGGAACCTGATG CTTTAACTTTGCTGGCCCCAGCCGCTGGAGAC ACAATCATATCTTTAGATTTTGGCAGCAACGA CACAGAAACTGATGACCAGCAACTTGAGGAA GTACCATTATATAATGATGTAATGCTCCCCTC ACCCAACGAAAAATTACAGAATATAAATTTG GCAATGTCTCCATTACCCACCGCTGAAACGCC AAAGCCACTTCGAAGTAGTGCTGACCCTGCA CTCAATCAAGAAGTTGCATTAAAATTAGAAC CAAATCCAGAGTCACTGGAACTTTCTTTTACC ATGCCCCAGATTCAGGATCAGACACCTAGTC CTTCCGATGGAAGCACTAGACAAAGTTCACC TGAGCCTAATAGTCCCAGTGAATATTGTTTTT ATGTGGATAGTGATATGGTCAATGAATTCAA GTTGGAATTGGTAGAAAAACTTTTTGCTGAAG ACACAGAAGCAAAGAACCCATTTTCTACTCA GGACACAGATTTAGACTTGGAGATGTTAGCT CCCTATATCCCAATGGATGATGACTTCCAGTT ACGTTCCTTCGATCAGTTGTCACCATTAGAAA GCAGTTCCGCAAGCCCTGAAAGCGCAAGTCC TCAAAGCACAGTTACAGTATTCCAGCAGACT CAAATACAAGAACCTACTGCTAATGCCACCA CTACCACTGCCACCACTGATGAATTAAAAAC AGTGACAAAAGACCGTATGGAAGACATTAAA ATATTGATTGCATCTCCATCTCCTACCCACAT ACATAAAGAAACTACTAGTGCCACATCATCA	

			CCATATAGAGATACTCAAAGTCGGACAGCCT CACCAAACAGAGCAGGAAAAGGAGTCATAG AACAGACAGAAAAATCTCATCCAAGAAGCCC TAACGTGTTATCTGTCGCTTTGAGTCAAAGAA CTACAGTTCCTGAGGAAGAACTAAATCCAAA GATACTAGCTTTGCAGAATGCTCAGAGAAAG CGAAAAATGGAACATGATGGTTCACTTTTTCA AGCAGTAGGAATTGGAACATTATTACAGCAG CCAGACGATCATGCAGCTACTACATCACTTTC TTGGAAACGTGTAAAAGGATGCAAATCTAGT GAACAGAATGGAATGGAGCAAAAGACAATTA TTTTAATACCCTCTGATTTAGCATGTAGACTG CTGGGGCAATCAATGGATGAAAGTGGATTAC CACAGCTGACCAGTTATGATTGTGAAGTTAAT GCTCCTATACAAGGCAGCAGAAACCTACTGC AGGGTGAAGAATTACTCAGAGCTTTGGATCA AGTTAACTGAGCTTTTTCTTAATTTCATTCCTT TTTTTGGACACTGGTGGCTCATTACCTAAAGC AGTCTATTTATATTTTCTACATCTAATTTTAGA AGCCTGGCTACAATACTGCACAAACTTGGTTA GTTCAATTTTGATCCCCTTTCTACTTAATTTAC ATTAATGCTCTTTTTTAGTATGTTCTTTAATGC TGGATCACAGACAGCTCATTTTCTCAGTTTTT TGGTATTTAAACCATTGCATTGCAGTAGCATC ATTTTAAAAAATGCACCTTTTTATTTATTTATT TTTGGCTAGGGAGTTTATCCCTTTTTCGAATT ATTTTTAAGAAGATGCCAATATAATTTTTGTA AGAAGGCAGTAACCTTTCATCATGATCATAG GCAGTTGAAAAATTTTTACACCTTTTTTTTCA CATTTTACATAAATAATAATGCTTTGCCAGCA GTACGTGGTAGCCACAATTGCACAATATATTT TCTTAAAAAATACCAGCAGTTACTCATGGAAT ATATTCTGCGTTTATAAAACTAGTTTTTAAGA AGAAATTTTTTTTGGCCTATGAAATTGTTAAA CCTGGAACATGACATTGTTAATCATATAATAA TGATTCTTAAATGCTGTATGGTTTATTATTTA AATGGGTAAAGCCATTTACATAATATAGAAA GATATGCATATATCTAGAAGG	
HIF1 -alpha	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	394997	GACAGGAGGATCACCCTCTTCGTCGCTTCGGC CAGTGTGTCGGGCTGGGCCCTGACAAGCCAC CTGAGGAGAGGCTCGGAGCCGGGCCCGGACC CCGGCGATTGCCGCCCGCTTCTCTCTAGTCTC ACGAGGGGTTTCCCGCCTCGCACCCCCACCTC TGGACTTGCCTTTCCTTCTCTTCTCCGCGTGTG GAGGGAGCCAGCGCTTAGGCCGGAGCGAGCC TGGGGGCCGCCCGCCGTGAAGACATCGCGGG GACCGATTCACCATGGAGGGCGCCGGCGGCG CGAACGACAAGAAAAATAGGATAAGTTCTGA ACGTCGAAAAGAAAAGTCTCGAGATGCAGCC AGATCTCGGCGAAGTAAAGAATCTGAAGTTT	6610

			TTTATGAGCTTGCTCATCAGTTGCCACTTCCA CATAATGTGAGTTCGCATCTTGATAAGGCCTC TGTGATGAGGCTTACCATCAGCTATTTGCGTG TGAGGAAACTTCTGGATGCTGGTGATTTGGAT ATTGAAGATGACATGAAAGCACAGATGAATT GCTTTTATTTGAAAGCCTTGGATGGTTTTGTT ATGGTTCTCACAGATGATGGTGACATGATTTA CATTTCTGATAATGTGAACAAATACATGGGAT TAACTCAGTTTGAACTAACTGGACACAGTGTG TTTGATTTTACTCATCCATGTGACCATGAGGA AATGAGAGAAATGCTTACACACAGAAATGGC CTTGTGAAAAAGGGTAAAGAACAAAACACAC AGCGAAGCTTTTTTCTCAGAATGAAGTGTACC CTAACTAGCCGAGGAAGAACTATGAACATAA AGTCTGCAACATGGAAGGTATTGCACTGCAC AGGCCACATTCACGTATATGATACCAACAGT AACCAACCTCAGTGTGGGTATAAGAAACCAC CTATGACCTGCTTGGTGCTGATTTGTGAACCC ATTCCTCACCCATCAAATATTGAAATTCCTTT AGATAGCAAGACTTTCCTCAGTCGACACAGC CTGGATATGAAATTTTCTTATTGTGATGAAAG AATTACCGAATTGATGGGATATGAGCCAGAA GAACTTTTAGGCCGCTCAATTTATGAATATTA TCATGCTTTGGACTCTGATCATCTGACCAAAA CTCATCATGATATGTTTACTAAAGGACAAGTC ACCACAGGACAGTACAGGATGCTTGCCAAAA GAGGTGGATATGTCTGGGTTGAAACTCAAGC AACTGTCATATATAACACCAAGAATTCTCAAC CACAGTGCATTGTATGTGTGAATTACGTTGTG AGTGGTATTATTCAGCACGACTTGATTTTCTC CCTTCAACAAACAGAATGTGTCCTTAAACCG GTTGAATCTTCAGATATGAAAATGACTCAGCT ATTCACCAAAGTTGAATCAGAAGATACAAGT AGCCTCTTTGACAAACTTAAGAAGGAACCTG ATGCTTTAACTTTGCTGGCCCCAGCCGCTGGA GACACAATCATATCTTTAGATTTTGGCAGCAA CGACACAGAAACTGATGACCAGCAACTTGAG GAAGTACCATTATATAATGATGTAATGCTCCC CTCACCCAACGAAAAATTACAGAATATAAAT TTGGCAATGTCTCCATTACCCACCGCTGAAAC GCCAAAGCCACTTCGAAGTAGTGCTGACCCT GCACTCAATCAAGAAGTTGCATTAAAATTAG AACCAAATCCAGAGTCACTGGAACTTTCTTTT ACCATGCCCCAGATTCAGGATCAGACACCTA GTCCTTCCGATGGAAGCACTAGACAAAGTTC ACCTGAGCCTAATAGTCCCAGTGAATATTGTT TTTATGTGGATAGTGATATGGTCAATGAATTC AAGTTGGAATTGGTAGAAAAACTTTTTGCTGA AGACACAGAAGCAAAGAACCCATTTTCTACT CAGGACACAGATTTAGACTTGGAGATGTTAG CTCCCTATATCCCAATGGATGATGACTTCCAG	

			TTACGTTCCTTCGATCAGTTGTCACCATTAGA AAGCAGTTCCGCAAGCCCTGAAAGCGCAAGT CCTCAAAGCACAGTTACAGTATTCCAGCAGA CTCAAATACAAGAACCTACTGCTAATGCCAC CACTACCACTGCCACCACTGATGAATTAAAA ACAGTGACAAAAGACCGTATGGAAGACATTA AAATATTGATTGCATCTCCATCTCCTACCCAC ATACATAAAGAAACTACTAGTGCCACATCAT CACCATATAGAGATACTCAAAGTCGGACAGC CTCACCAAACAGAGCAGGAAAAGGAGTCATA GAACAGACAGAAAAATCTCATCCAAGAAGCC CTAACGTGTTATCTGTCGCTTTGAGTCAAAGA ACTACAGTTCCTGAGGAAGAACTAAATCCAA AGATACTAGCTTTGCAGAATGCTCAGAGAAA GCGAAAAATGGAACATGATGGTTCACTTTTTC AAGCAGTAGGAATTGGAACATTATTACAGCA GCCAGACGATCATGCAGCTACTACATCACTTT CTTGGAAACGTGTAAAAGGATGCAAATCTAG TGAACAGAATGGAATGGAGCAAAAGACAATT ATTTTAATACCCTCTGATTTAGCATGTAGACT GCTGGGGCAATCAATGGATGAAAGTGGATTA CCACAGCTGACCAGTTATGATTGTGAAGTTAA TGCTCCTATACAAGGCAGCAGAAACCTACTG CAGGGTGAAGAATTACTCAGAGCTTTGGATC AAGTTAACTGAGCTTTTTCTTAATTTCATTCCT TTTTTTGGACACTGGTGGCTCATTACCTAAAG CAGTCTATTTATATTTTCTACATCTAATTTTAG AAGCCTGGCTACAATACTGCACAAACTTGGTT AGTTCAATTTTGATCCCCTTTCTACTTAATTTA CATTAATGCTCTTTTTTAGTATGTTCTTTAATG CTGGATCACAGACAGCTCATTTTCTCAGTTTT TTGGTATTTAAACCATTGCATTGCAGTAGCAT CATTTTAAAAAATGCACCTTTTTATTTATTTAT TTTTGGCTAGGGAGTTTATCCCTTTTTCGAATT ATTTTTAAGAAGATGCCAATATAATTTTTGTA AGAAGGCAGTAACCTTTCATCATGATCATAG GCAGTTGAAAAATTTTTACACCTTTTTTTTCA CATTTTACATAAATAATAATGCTTTGCCAGCA GTACGTGGTAGCCACAATTGCACAATATATTT TCTTAAAAAATACCAGCAGTTACTCATGGAAT ATATTCTGCGTTTATAAAACTAGTTTTTAAGA AGAAATTTTTTTTGGCCTATGAAATTGTTAAA CCTGGAACATGACATTGTTAATCATATAATAA TGATTCTTAAATGCTGTATGGTTTATTATTTA AATGGGTAAAGCCATTTACATAATATAGAAA GATATGCATATATCTAGAAGGTATGTGGCATT TATTTGGATAAAATTCTCAATTCAGAGAAATC ATCTGATGTTTCTATAGTCACTTTGCCAGCTC AAAAGAAAACAATACCCTATGTAGTTGTGGA AGTTTATGCTAATATTGTGTAACTGATATTAA ACCTAAATGTTCTGCCTACCCTGTTGGTATAA	

			AGATATTTTGAGCAGACTGTAAACAAGAAAA AAAAAATCATGCATTCTTAGCAAAATTGCCTA GTATGTTAATTTGCTCAAAATACAATGTTTGA TTTTATGCACTTTGTCGCTATTAACATCCTTTT TTTCATGTAGATTTCAATAATTGAGTAATTTT AGAAGCATTATTTTAGGAATATATAGTTGTCA CAGTAAATATCTTGTTTTTTCTATGTACATTGT ACAAATTTTTCATTCCTTTTGCTCTTTGTGGTT GGATCTAACACTAACTGTATTGTTTTGTTACA TCAAATAAACATCTTCTGTGGACCAGG	

Table 28. Peptide sequences for additional targets for titration experiments

Target	Target Description	ENSP ID	Protein Sequence	SEQ ID NO
HIF2-alpha	hypoxia inducible factor 2, alpha subunit; endothelial PAS domain protein 1	263734	MTADKEKKRSSSERRKEKSRDAARCRRSKETE VFYELAHELPLPHSVSSHLDKASIMRLAISFLRT HKLLSSVCSENESEAEADQQMDNLYLKALEGFI AVVTQDGDMIFLSENISKFMGLTQVELTGHSIF DFTHPCDHEEIRENLSLKNGSGFGKKSKDMSTE RDFFMRMKCTVTNRGRTVNLKSATWKVLHCT GQVKVYNNCPPHNSLCGYKEPLLSCLIIMCEPIQ HPSHMDIPLDSKTFLSRHSMDMKFTYCDDRITE LIGYHPEELLGRSAYEFYHALDSENMTKSHQNL CTKGQVVSGQYRMLAKHGGYVWLETQGTVIY NPRNLQPQCIMCVNYVLSEIEKNDVVFSMDQTE SLFKPHLMAMNSIFDSSGKGAVSEKSNFLFTKL KEEPEELAQLAPTPGDAIISLDFGNQNFEESSAY GKAILPPSQPWATELRSHSTQSEAGSLPAFTVPQ AAAPGSTTPSATSSSSSCSTPNSPEDYYTSLDND LKIEVIEKLFAMDTEAKDQCSTQTDFNELDLET LAPYIPMDGEDFQLSPICPEERLLAENPQSTPQH CFSAMTNIFQPLAPVAPHSPFLLDKFQQQLESKK TEPEHRPMSSIFFDAGSKASLPPCCGQASTPLSS MGGRSNTQWPPDPPLHFGPTKWAVGDQRTEFL GAAPLGPPVSPPHVSTFKTRSAKGFGARGPDVL SPAMVALSNKLKLKRQLEYEEQAFQDLSGGDP PGGSTSHLMWKRMKNLRGGSCPLMPDKPLSAN VPNDKFTQNPMRGLGHPLRHLPLPQPPSAISPGE NSKSRFPPQCYATQYQDYSLSSAHKVSGMASR LLGPSFESYLLPELTRYDCEVNVPVLGSSTLLQG GDLLRALDQAT	6611
pVHL	von Hippel-Lindau tumor	256474	MPRRAENWDEAEVGAEEAGVEEYGPEEDGGE ESGAEESGPEESGPEELGAEEEMEAGRPRPVLRS VNSREPSQVIFCNRSPRVVLPVWLNFDGEPQPY PTLPPGTGRRIHSYRGHLWLFRDAGTHDGLLVN	6612

	suppressor		QTELFVPSLNVDGQPIFANITLPVYTLKERCLQV VRSLVKPENYRRLDIVRSLYEDLEDHPNVQKDL ERLTQERIAHQRMGD	
pVHL	von Hippel-Lindau tumor suppressor	344757	MPRRAENWDEAEVGAEEAGVEEYGPEEDGGE ESGAEESGPEESGPEELGAEEEMEAGRPRPVLRS VNSREPSQVIFCNRSPRVVLPVWLNFDGEPQPY PTLPPGTGRRIHSYRVYTLKERCLQVVRSLVKP ENYRRLDIVRSLYEDLEDHPNVQKDLERLTQER IAHQRMGD	6613
pVHL	von Hippel-Lindau tumor suppressor	395399	MPRRAENWDEAEVGAEEAGVEEYGPEEDSYR GHLWLFRDAGTHDGLLVNQTELFVPSLNVDGQ PIFANITLPVYTLKERCLQVVRSLVKPENYRRLD IVRSLYEDLEDHPNVQKDLERLTQERIAHQRMG D	6614
HIF1-alpha	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	451696	MRLTISYLRVRKLLDAGDLDIEDDMKAQMNCF YLKALDGFVMVLTDDGDMIYISDNVNKYMGL TQFELTGHSVFDFTHPCDHEEMREMLTHRNGL VKKGKEQNTQRSFFLRMKCTLTSRGRTMNIKS ATWKVLHCTGHIHVYDTNSNQPQCGYKKPPMT CLVLICEPIPHPSNIEIPLDSKTFLSRHSLDMKFSY CDERITELMGYEPEELLGRSIYEYYHALDSDHL TKTHHDMFTKGQVTTGQYRMLAKRGGYVWV ETQATVIYNTKNSQPQCIVCVNYVVSGIIQHDLI FSLQQTECVLKPVESSDMKMTQLFTKVESEDTS SLFDKLKKEPDALTLLAPAAGDTIISLDFGSNDT ETDDQQLEEVPLYNDVMLPSPNEKLQNINLAM SPLPTAETPKPLRSSADPALNQEVALKLEPNPES LELSFTMPQIQDQTPSPSDGSTRQSSPEPNSPSEY CFYVDSDMVNEFKLELVEKLFAEDTEAKNPFST QDTDLDLEMLAPYIPMDDDFQLRSFDQLSPLES SSASPESASPQSTVTVFQQTQIQEPTANATTTTA TTDELKTVTKDRMEDIKILIASPSPTHIHKETTSA TSSPYRDTQSRTASPNRAGKGVIEQTEKSHPRSP NVLSVALSQRTTVPEEELNPKILALQNAQRKRK MEHDGSLFQAVGIGTLLQQPDDHAATTSLSWK RVKGCKSSEQNGMEQKTIILIPSDLACRLLGQS MDESGLPQLTSYDCEVNAPIQGSRNLLQGEELL RALDQVN	6615
HIF1-alpha	hypoxia inducible factor 1, alpha subunit	378446	MEGAGGANDKKNRISSERRKEKSRDAARSRRS KESEVFYELAHQLPLPHNVSSHLDKASVMRLTI SYLRVRKLLDAGDLDIEDDMKAQMNCFYLKAL DGFVMVLTDDGDMIYISDNVNKYMGLTQFELT GHSVFDFTHPCDHEEMREMLTHRNGLVKKGKE	6616

	(basic helix-loop-helix transcription factor)		QNTQRSFFLRMKCTLTSRGRTMNIKSATWKVL HCTGHIHVYDTNSNQPQCGYKKPPMTCLVLICE PIPHPSNIEIPLDSKTFLSRHSLDMKFSYCDERITE LMGYEPEELLGRSIYEYYHALDSDHLTKTHHD MFTKGQVTTGQYRMLAKRGGYVWVETQATVI YNTKNSQPQCIVCVNYVVSGIIQHDLIFSLQQTE CVLKPVESSDMKMTQLFTKVESEDTSSLFDKLK KEPDALTLLAPAAGDTIISLDFGSNDTETDDQQL EEVPLYNDVMLPSPNEKLQNINLAMSPLPTAET PKPLRSSADPALNQEVALKLEPNPESLELSFTMP QIQDQTPSPSDGSTRQSSPEPNSPSEYCFYVDSD MVNEFKLELVEKLFAEDTEAKNPFSTQDTDLDL EMLAPYIPMDDDFQLRSFDQLSPLESSSASPESA SPQSTVTVFQQTQIQEPTANATTTTATTDELKTV TKDRMEDIKILIASPSPTHIHKETTSATSSPYRDT QSRTASPNRAGKGVIEQTEKSHPRSPNVLSVAL SQRTTVPEEELNPKILALQNAQRKRKMEHDGSL FQAVGIGTLLQQPDDHAATTSLSWKRVKGCKS SEQNGMEQKTIILIPSDLACRLLGQSMDESGLPQ LTSYDCEVNAPIQGSRNLLQGEELLRALDQVN	

Materials for Examples 27-33

[001029] Table 29 describes the modified mRNA sequences described in Examples 27-33.

Table 29

Target	mRNA Sequence (polyA tail and 5'cap not shown in sequence)	SEQ ID NO
Apoptosis-inducing factor short (AIFsh)	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCC ACCAUGGAAAAAGUCAGACGAGAGGGGGUUAAGGUGAUGCCCAA UGCUAUUGUGCAAUCCGUUGGAGUCAGCAGUGGCAAGUUACUUA UCAAGCUGAAAGACGGCAGGAAGGUAGAAACUGACCACAUAGUG GCAGCUGUGGGCCUGGAGCCCAAUGUUGAGUUGGCCAAGACUGG UGGCCUGGAAAUAGACUCAGAUUUUGGUGGCUUCCGGGUAAAUG CAGAGCUACAAGCACGCUCUAACAUCUGGGUGGCAGGAGAUGCU GCAUGCUUCUACGAUAUAAAGUUGGGAAGGAGGCGGGUAGAGCA CCAUGAUCACGCUGUUGUGAGUGGAAGAUUGGCUGGAGAAAAUA UGACUGGAGCUGCUAAGCCGUACUGGCAUCAGUCAAUGUUCUGG AGUGAUUUGGGCCCCGAUGUUGGCUAUGAAGCUAUUGGUCUUGU GGACAGUAGUUUGCCCACAGUUGGUGUUUUUGCAAAAGCAACUG CACAAGACAACCCCAAAUCUGCCACAGAGCAGUCAGGAACUGGUA UCCGAUCAGAGAGUGAGACAGAGUCCGAGGCCUCAGAAAUUACU AUUCCUCCCAGCACCCCGGCAGUUCCACAGGCUCCCGUCCAGGGG GAGGACUACGGCAAAGGUGUCAUCUUCUACCUCAGGGACAAAGU GGUCGUGGGGAUUGUGCUAUGGAACAUCUUUAACCGAAUGCCAA UAGCAAGGAAGAUCAUUAAGGACGGUGAGCAGCAUGAAGAUCUC AAUGAAGUAGCCAAACUAUUCAACAUUCAUGAAGACUGAUAAUA	6617

	GGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCC CCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCGUGGUCUUUG AAUAAAGUCUGAGUGGGCGGC	
Siah E3 ubiquitin protein ligase 1 (SIAH1)	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCC ACCAUGAGCCGUCAGACUGCUACAGCAUUACCUACCGGUACCUCG AAGUGUCCACCAUCCCAGAGGGUGCCUGCCCUGACUGGCACAACU GCAUCCAACAAUGACUUGGCGAGUCUUUUUGAGUGUCCAGUCUG CUUUGACUAUGUGUUACCGCCCAUUCUUCAAUGUCAGAGUGGCC AUCUUGUUUGUAGCAACUGUCGCCCAAAGCUCACAUGUUGUCCA ACUUGCCGGGGCCCUUUGGGAUCCAUUCGCAACUUGGCUAUGGA GAAAGUGGCUAAUUCAGUACUUUUCCCCUGUAAAUAUGCGUCUU CUGGAUGUGAAAUAACUCUGCCACACACAGAAAAAGCAGACCAU GAAGAGCUCUGUGAGUUUAGGCCUUAUUCCUGUCCGUGCCCUGG UGCUUCCUGUAAAUGGCAAGGCUCUCUGGAUGCUGUAAUGCCCC AUCUGAUGCAUCAGCAUAAGUCCAUUACAACCCUACAGGGAGAG GAUAUAGUUUUUCUUGCUACAGACAUUAAUCUUCCUGGUGCUGU UGACUGGGUGAUGAUGCAGUCCUGUUUUGGCUUUCACUUCAUGU UAGUCUUAGAGAAACAGGAAAAAUACGAUGGUCACCAGCAGUUC UUCGCAAUCGUACAGCUGAUAGGAACACGCAAGCAAGCUGAAAA UUUUGCUUACCGACUUGAGCUAAAUGGUCAUAGGCGACGAUUGA CUUGGGAAGCGACUCCUCGAUCUAUUCAUGAAGGAAUUGCAACA GCCAUUAUGAAUAGCGACUGUCUAGUCUUUGACACCAGCAUUGC ACAGCUUUUUGCAGAAAAUGGCAAUUUAGGCAUCAAUGUAACUA UUUCCAUGUGUUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUU CUUGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCACC CGUACCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC	6618
Constitively active (C.A. caspase 3 (also known as reverse caspase 3 (Rev-Caspase 3))	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCC ACCAUGAUUGAGACAGACAGUGGUGUUGAUGAUGACAUGGCGUG UCAUAAAAUACCAGUGGAGGCCGACUUCUUGUAUGCAUACUCCA CAGCACCUGGUUAUUAUUCUUGGCGAAAUUCAAAGGAUGGCUCC UGGUUCAUCCAGUCGCUUUGUGCCAUGCUGAAACAGUAUGCCGA CAAGCUUGAAUUUAUGCACAUUCUUACCCGGGUUAACCGAAAGG UGGCAACAGAAUUUGAGUCCUUUUCCUUUGACGCUACUUUUC^ U GCAAAGAAACAGAUUCCAUGUAUUGUUUCCAUGCUCACAAAAGA ACUCUAUUUUUAUCACGAUGAAGUUGAUGGGGGAUCCCCCAUGG AGAACACUGAAAACUCAGUGGAUUCAAAAUCCAUUAAAAAUUUG GAACCAAAGAUCAUACAUGGAAGCGAAUCAAUGGACUCUGGAAU AUCCCUGGACAACAGUUAUAAAAUGGAUUAUCCUGAGAUGGGUU UAUGUAUAAUAAUUAAUAAUAAGAAUUUUCAUAAGAGCACUGGA AUGACAUCUCGGUCUGGUACAGAUGUCGAUGCAGCAAACCUCAG GGAAACAUUCAGAAACUUGAAAUAUGAAGUCAGGAAUAAAAAUG AUCUUACACGUGAAGAAAUUGUGGAAUUGAUGCGUGAUGUUUCU AAAGAAGAUCACAGCAAAAGGAGCAGUUUUGUUUGUGUGCUUCU GAGCCAUGGUGAAGAAGGAAUAAUUUUUGGAACAAAUGGACCUG UUGACCUGAAAAAAAUAACAAACUUUUUCAGAGGGGAUCGUUGU AGAAGUCUAACUGGAAAACCCAAACUUUUCAUUAUUCAGGCCUG CCGUGGUACAGAACUGGACUGUGGCAUUGAGACAGACUGAUAAU AGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCC CCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCGUGGUCUUU GAAUAAAGUCUGAGUGGGCGGC	6619
Granulysin	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCC ACCAUGGCAACUUGGGCCCUGCUGCUUCUUGCAGCCAUGUUGCUC GGAAAUCCUGGUCUGGUGUUUUCGCGCCUUUCACCGGAGUACUA CGAUCUCGCUCGCGCACAUCUGCGCGACGAGGAGAAGUCGUGCCC AUGUCUCGCACAAGAAGGGCCACAGGGUGACCUUUUGACCAAGA	6620

	CGCAAGAACUUGGCAGGGACUACCGAACCUGUCUGACCAUCGUGC AAAAGCUGAAGAAAAUGGUCGAUAAACCUACCCAAAGAAGCGUG UCCAACGCAGCGACUCGGGUGUGCCGGACUGGCAGAUCCAGAUG GCGGGAUGUGUGUAGAAACUUCAUGAGAAGGUACCAGAGCCGUG UUACUCAGGGACUGGUCGCGGGAGAAACUGCCCAACAGAUUUGC GAAGAUCUGCGACUCUGUAUUCCUUCAACCGGACCCCUUUGAUA AUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUC CCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCGUGGUCUU UGAAUAAAGUCUGAGUGGGCGGC	
MYC inhibitor **D**	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCC ACCAUGACCGAAGAAAACGUCAAGAGAAGAACCCAUAAUGUCCU CGAGCGCCAGCGGCGCAAUGAGCUCAAGCGCAGCUUCUUUGCACU CAGGGACCAAAUUCCAGAGUUGGAGAACAACGAAAAGGCCCCGA AGGUGGUGAUCCUUAAGAAGGCGACUGCCUACAUCCUGUCGGUG CAGGCUGAGACUCAAAAGCUGAUCUCCGAAAUCGAUCUGCUCCG GAAACAGAACGAACAACUGAAACACAAACUGGAACAGCUGCGGA AUUCAUGCUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUU GCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGU ACCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC	6621

Example 27. Detection of Apoptosis-Inducing Factor short Protein: Western Blot

[001030] CD1 mice (Harlan Laboratories, South Easton, MA) were administered intravenously lipolexed apoptosis-inducing factor short (AIFsh) modified mRNA (mRNA sequence shown in Table 29; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) fully modified with 5-methylcytidine and pseudouridine (5mC/pU), fully modified with 5-methylcytidine and 1-methylpseudouridine (5mC/lmpU), 25% of uridine modified with 2-thiouridine and 25% of cytidine modified with 5-methylcytidine (s2U and 5mC), fully modified with pseudouridine (pU) or fully modified with 1-methylpseudouridine (ImpU). The mice were administered a dose of 2 ug of mRNA complexed with 2ul Lipofectamine 2000 (LifeTechnologies, Grand Island, NY) in lOOul sterile basal DMEM medium (w/o additives, LifeTechnologies, Grand Island, NY).

[001031] After 6 hours, the animals were sacrificed and serum & spleen are taken. Spleens were transferred to 6-well plates and kept on ice in presence of 1ml PBS. One spleen was cut with a scalpel several times and with a rubber cell scraper splenocytes were squeezed out until the PBS turns turbid due to cell release.

[001032] Leaving fibrous components behind, the cells were transferred to a lOOum cell strainer (BD Biosciences, San Jose, CA) sitting on a 12-well cell culture plate. By gravity the cells passed through the cell strainer and were collected beneath in the 12-well culture dish. 1 ml of PBS was transferred with the free-floating splenocytes to an Eppendorf

tube and spun for 5 min at 2000 rpm. The PBS was discarded and the cell pellet combined with 500 ul fresh PBS. The spenocytes were resuspended by brief vortexing for 5 mins at 2000 rpm. The PBS was discarded and 1ml BD Pharmlyse was added to the cell pellet. The splenocytes were resuspended by brief vortexing. The cells were incubated at room temperature for 3 minutes and then spun at 200 rpm for 5 minutes. The cells were washed twice with 500 ul PBS and spun as described above. The cells were resuspended with 500 ul of PBS and spun as described.

[001033] 250 ul of splenocytes were combined with lx Pharmlyse buffer and vortexed briefly or resuspended with a pipet and then spun for 2 minutes at 2000 rpm.

[001034] In one tube, resuspend cell pellet in 500ul RIPA buffer with protease inhibitor cocktail for mammalian cells (BostonBioproducts, Ashland, MA) and freeze lysate or continue with BCA assay immediately. In a second tube, add 250ul FACS staining kit fixation solution (4% formaldehyde; R and D Systems, Minneapolis, MN) and then incubate for 10 minutes at room temperature. The cells were washed twice with 500 ul PBS and spun as described above. The cell pellet was resuspended in 500 PBS and stored at 4°C.

[001035] Protein lysates were loaded on NuPage SDS-PAGE system (chambers and power supply) with 1.5mm ready-to-use Bis-Tris gels and 4-12% acrylamide gradient with MOPS-buffer as running aid (all Life Technologies, Grand Island, NY). Each lysate sample was prepared to 40ul final volume. This sample contained 25ug protein lysate in variable volume, RIPA buffer to make up volume to 26ul, 4ul of lOx reducing agent and lOul 4x SDS loading buffer (both from Life Technologies, Grand Island, NY). Samples were heated at 95oC for 5min and loaded on the gel. Standard settings were chosen by the manufacturer, 200V, 120mA and max. 25W. Run time was 60min, but no longer than running dye reaching the lower end of the gel.

[001036] After the run was terminated, the plastic case was cracked and the encased gel transferred to a ready-to-use nitrocellulose membrane kit and power supply (iBLOT; LifeTechnologies, Grand Island, NY). Using default settings, the protein lysate was transferred by high Ampere electricity from the gel to the membrane.

[001037] After the transfer, the membranes were incubated in 5% BSA in IX TBS for 15 minutes then in 5% BSA in IX TBS + 0.1 % Tween for another 15 minutes. Primary

antibodies (AIFsh rabbit polyclonal antibody; Abeam, Cambridge, MA) against AIFsh proteins were applied in 3ml of 5% BSA in IX TBS solution at a 1:500 to 1:2000 dilution for 3 hours at room temperature and gentle agitation on an orbital shaker. Membranes are washed 3 times with IX TBS/0.1 % Tween, 5 minutes each time with gentle agitation. The secondary antibody (Goat anti-rabbit HRP conjugate; Abeam, Cambridge, MA) was conjugated to horse radish peroxidase and binds to the primary antibody antibodies. The secondary antibody was diluted of 1:1000 to 1:5000 in 5% BSA in IX TBS and incubated for 3 hrs at RT.

[001038] At the end of incubation time, the membranes were washed 3 times with IX TBS/0.1 % Tween, 5 minutes each time with gentle agitation. The membranes were developed in 5ml Pierce WestPico Chemiluminescent Subtrate (Thermo Fisher, Rockford, IL) as directed.

[001039] As shown in Figures 3A and 3B the Western Blot detected protein around the expected size of 60 kd for each of the 2 samples evaluated for each chemistry.

Example 28. Detection of Siah E3 ubiquitin protein ligase 1 Protein: Western Blot

[001040] CD1 mice (Harlan Laboratories, South Easton, MA) were administered intravenously lipolexed siah E3 ubiquitin protein ligase 1 (SIAH1) modified mRNA (mRNA sequence shown in SEQ ID NO. 6618 (Table 29); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) fully modified with 5-methylcytidine and pseudouridine (5mC/pU), fully modified with 5-methylcytidine and 1-methylpseudouridine (5mC/lmpU), 25% of uridine modified with 2-thiouridine and 25% of cytidine modified with 5-methylcytidine (s2U and 5mC), fully modified with pseudouridine (pU) or fully modified with 1-methylpseudouridine (ImpU). The mice were administered a dose of 2 ug of mRNA complexed with 2ul Lipofectamine 2000 (LifeTechnologies, Grand Island, NY) in lOOul sterile basal DMEM medium (w/o additives, LifeTechnologies, Grand Island, NY).

[001041] After 6 hours, the animals were sacrificed and serum & spleen are taken. Spleens were transferred to 6-well plates and kept on ice in presence of 1ml PBS. One spleen was cut with a scalpel several times and with a rubber cell scraper splenocytes were squeezed out until the PBS turns turbid due to cell release.

[001042] Leaving fibrous components behind, the cells were transferred to a lOOum cell strainer (BD Biosciences, San Jose, CA) sitting on a 12-well cell culture plate. By gravity the cells passed through the cell strainer and were collected beneath in the 12-well culture dish. 1 ml of PBS was transferred with the free-floating splenocytes to an Eppendorf tube and spun for 5 min at 2000 rpm. The PBS was discarded and the cell pellet combined with 500 ul fresh PBS. The spenocytes were resuspended by brief vortexing for 5 mins at 2000 rpm. The PBS was discarded and 1ml BD Pharmlyse was added to the cell pellet. The splenocytes were resuspended by brief vortexing. The cells were incubated at room temperature for 3 minutes and then spun at 200 rpm for 5 minutes. The cells were washed twice with 500 ul PBS and spun as described above. The cells were resuspended with 500 ul of PBS and spun as described.

[001043] 250 ul of splenocytes were combined with lx Pharmlyse buffer and vortexed briefly or resuspended with a pipet and then spun for 2 minutes at 2000 rpm.

[001044] In one tube, resuspend cell pellet in 500ul RIPA buffer with protease inhibitor cocktail for mammalian cells (BostonBioproducts, Ashland, MA) and freeze lysate or continue with BCA assay immediately. In a second tube, add 250ul FACS staining kit fixation solution (4% formaldehyde; R and D Systems, Minneapolis, MN) and then incubate for 10 minutes at room temperature. The cells were washed twice with 500 ul PBS and spun as described above. The cell pellet was resuspended in 500 PBS and stored at 4°C.

[001045] Protein lysates were loaded on NuPage SDS-PAGE system (chambers and power supply) with 1.5mm ready-to-use Bis-Tris gels and 4-12% acrylamide gradient with MOPS-buffer as running aid (all Life Technologies, Grand Island, NY). Each lysate sample was prepared to 40ul final volume. This sample contained 25ug protein lysate in variable volume, RIPA buffer to make up volume to 26ul, 4ul of lOx reducing agent and lOul 4x SDS loading buffer (both from Life Technologies, Grand Island, NY). Samples were heated at 95oC for 5min and loaded on the gel. Standard settings were chosen by the manufacturer, 200V, 120mA and max. 25W. Run time was 60min, but no longer than running dye reaching the lower end of the gel.

[001046] After the run was terminated, the plastic case was cracked and the encased gel transferred to a ready-to-use nitrocellulose membrane kit and power supply (iBLOT;

LifeTechnologies, Grand Island, NY). Using default settings, the protein lysate was transferred by high Ampere electricity from the gel to the membrane.

[001047] After the transfer, the membranes were incubated in 5% BSA in IX TBS for 15 minutes then in 5% BSA in IX TBS + 0.1 % Tween for another 15 minutes. Primary antibodies (SIAH1 rabbit polyclonal antibody; Abeam, Cambridge, MA) against SIAH1 proteins were applied in 3ml of 5% BSA in IX TBS solution at a 1:500 to 1:2000 dilution for 3 hours at room temperature and gentle agitation on an orbital shaker. Membranes are washed 3 times with IX TBS/0.1 % Tween, 5 minutes each time with gentle agitation. The secondary antibody (Goat anti-rabbit HRP conjugate; Abeam, Cambridge, MA) was conjugated to horse radish peroxidase and binds to the primary antibody antibodies. The secondary antibody was diluted of 1:1000 to 1:5000 in 5% BSA in IX TBS and incubated for 3 hrs at RT.

[001048] At the end of incubation time, the membranes were washed 3 times with IX TBS/0.1 % Tween, 5 minutes each time with gentle agitation. The membranes were developed in 5ml Pierce WestPico Chemiluminescent Subtrate (Thermo Fisher, Rockford, IL) as directed.

[001049] As shown in Figures 4A and 4B the Western Blot detected protein around the expected size of 31 kd for each of the 2 samples evaluated for each chemistry.

Example 29. Detection of Reverse Caspase 3 Protein: Western Blot

[001050] CD1 mice (Harlan Laboratories, South Easton, MA) were administered intravenously lipolexed constitutively active (C.A.) caspase 3 (also known as Reverse-Caspase 3 or Rev-Caspase 3) modified mRNA (mRNA sequence shown in SEQ ID NO. 6619 (Table 29); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) fully modified with 5-methylcytidine and pseudouridine (5mC/pU), fully modified with 5-methylcytidine and 1-methylpseudouridine (5mC/lmpU), 25% of uridine modified with 2-thiouridine and 25% of cytidine modified with 5-methylcytidine (s2U and 5mC), fully modified with pseudouridine (pU) or fully modified with 1-methylpseudouridine (ImpU). The mice were administered a dose of 2 ug of mRNA complexed with 2ul Lipofectamine 2000 (LifeTechnologies, Grand Island, NY) in lOOul sterile basal DMEM medium (w/o additives, LifeTechnologies, Grand Island, NY).

[001051] After 6 hours, the animals were sacrificed and serum & spleen are taken. Spleens were transferred to 6-well plates and kept on ice in presence of 1ml PBS. One spleen was cut with a scalpel several times and with a rubber cell scraper splenocytes were squeezed out until the PBS turns turbid due to cell release.

[001052] Leaving fibrous components behind, the cells were transferred to a lOOum cell strainer (BD Biosciences, San Jose, CA) sitting on a 12-well cell culture plate. By gravity the cells passed through the cell strainer and were collected beneath in the 12-well culture dish. 1 ml of PBS was transferred with the free-floating splenocytes to an Eppendorf tube and spun for 5 min at 2000 rpm. The PBS was discarded and the cell pellet combined with 500 ul fresh PBS. The spenocytes were resuspended by brief vortexing for 5 mins at 2000 rpm. The PBS was discarded and 1ml BD Pharmlyse was added to the cell pellet. The splenocytes were resuspended by brief vortexing. The cells were incubated at room temperature for 3 minutes and then spun at 200 rpm for 5 minutes. The cells were washed twice with 500 ul PBS and spun as described above. The cells were resuspended with 500 ul of PBS and spun as described.

[001053] 250 ul of splenocytes were combined with lx Pharmlyse buffer and vortexed briefly or resuspended with a pipet and then spun for 2 minutes at 2000 rpm.

[001054] In one tube, resuspend cell pellet in 500ul RIPA buffer with protease inhibitor cocktail for mammalian cells (BostonBioproducts, Ashland, MA) and freeze lysate or continue with BCA assay immediately. In a second tube, add 250ul FACS staining kit fixation solution (4% formaldehyde; R and D Systems, Minneapolis, MN) and then incubate for 10 minutes at room temperature. The cells were washed twice with 500 ul PBS and spun as described above. The cell pellet was resuspended in 500 PBS and stored at 4°C.

[001055] Protein lysates were loaded on NuPage SDS-PAGE system (chambers and power supply) with 1.5mm ready-to-use Bis-Tris gels and 4-12% acrylamide gradient with MOPS-buffer as running aid (all Life Technologies, Grand Island, NY). Each lysate sample was prepared to 40ul final volume. This sample contained 25ug protein lysate in variable volume, RIPA buffer to make up volume to 26ul, 4ul of lOx reducing agent and lOul 4x SDS loading buffer (both from Life Technologies, Grand Island, NY). Samples were heated at 95oC for 5min and loaded on the gel. Standard settings were chosen by

the manufacturer, 200V, 120mA and max. 25W. Run time was 60min, but no longer than running dye reaching the lower end of the gel.

[001056] After the run was terminated, the plastic case was cracked and the encased gel transferred to a ready-to-use nitrocellulose membrane kit and power supply (iBLOT; LifeTechnologies, Grand Island, NY). Using default settings, the protein lysate was transferred by high Ampere electricity from the gel to the membrane.

[001057] After the transfer, the membranes were incubated in 5% BSA in IX TBS for 15 minutes then in 5% BSA in IX TBS + 0.1 % Tween for another 15 minutes. Primary antibodies (Caspase 3 rabbit polyclonal antibody; Abeam, Cambridge, MA) against target proteins were applied in 3ml of 5% BSA in IX TBS solution at a 1:500 to 1:2000 dilution for 3 hours at room temperature and gentle agitation on an orbital shaker. Membranes are washed 3 times with IX TBS/0.1 % Tween, 5 minutes each time with gentle agitation. The secondary antibody (Goat anti-rabbit HRP conjugate; Abeam, Cambridge, MA) was conjugated to horse radish peroxidase and binds to the primary antibody antibodies. The secondary antibody was diluted of 1:1000 to 1:5000 in 5% BSA in IX TBS and incubated for 3 hrs at RT.

[001058] At the end of incubation time, the membranes were washed 3 times with IX TBS/0.1 % Tween, 5 minutes each time with gentle agitation. The membranes were developed in 5ml Pierce WestPico Chemiluminescent Subtrate (Thermo Fisher, Rockford, IL) as directed.

[001059] As shown in Figures 5A and 5B the Western Blot detected protein around the expected size of 32 kd for each of the 2 samples evaluated for each chemistry.

Example 30. Detection of Granulysin Protein: Western Blot

[001060] CD1 mice (Harlan Laboratories, South Easton, MA) were administered intravenously lipolexed granulysin mRNA (mRNA sequence shown in SEQ ID NO. 6620 (Table 29); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) fully modified with 5-methylcytidine and pseudouridine (5mC/pU), fully modified with 5-methylcytidine and 1-methylpseudouridine (5mC/lmpU), 25% of uridine modified with 2-thiouridine and 25% of cytidine modified with 5-methylcytidine (s2U and 5mC), fully modified with pseudouridine (pU) or fully modified with 1-methylpseudouridine (ImpU). The mice were administered a dose of 2 ug of mRNA

complexed with 2ul Lipofectamine 2000 (LifeTechnologies, Grand Island, NY) in lOOul sterile basal DMEM medium (w/o additives, LifeTechnologies, Grand Island, NY).

[001061] After 6 hours, the animals were sacrificed and serum & spleen are taken. Spleens were transferred to 6-well plates and kept on ice in presence of 1ml PBS. One spleen was cut with a scalpel several times and with a rubber cell scraper splenocytes were squeezed out until the PBS turns turbid due to cell release.

[001062] Leaving fibrous components behind, the cells were transferred to a lOOum cell strainer (BD Biosciences, San Jose, CA) sitting on a 12-well cell culture plate. By gravity the cells passed through the cell strainer and were collected beneath in the 12-well culture dish. 1 ml of PBS was transferred with the free-floating splenocytes to an Eppendorf tube and spun for 5 min at 2000 rpm. The PBS was discarded and the cell pellet combined with 500 ul fresh PBS. The spenocytes were resuspended by brief vortexing for 5 mins at 2000 rpm. The PBS was discarded and 1ml BD Pharmlyse was added to the cell pellet. The splenocytes were resuspended by brief vortexing. The cells were incubated at room temperature for 3 minutes and then spun at 200 rpm for 5 minutes. The cells were washed twice with 500 ul PBS and spun as described above. The cells were resuspended with 500 ul of PBS and spun as described.

[001063] 250 ul of splenocytes were combined with lx Pharmlyse buffer and vortexed briefly or resuspended with a pipet and then spun for 2 minutes at 2000 rpm.

[001064] In one tube, resuspend cell pellet in 500ul RIPA buffer with protease inhibitor cocktail for mammalian cells (BostonBioproducts, Ashland, MA) and freeze lysate or continue with BCA assay immediately. In a second tube, add 250ul FACS staining kit fixation solution (4% formaldehyde; R and D Systems, Minneapolis, MN) and then incubate for 10 minutes at room temperature. The cells were washed twice with 500 ul PBS and spun as described above. The cell pellet was resuspended in 500 PBS and stored at 4°C.

[001065] Protein lysates were loaded on NuPage SDS-PAGE system (chambers and power supply) with 1.5mm ready-to-use Bis-Tris gels and 4-12% acrylamide gradient with MOPS-buffer as running aid (all Life Technologies, Grand Island, NY). Each lysate sample was prepared to 40ul final volume. This sample contained 25ug protein lysate in variable volume, RIPA buffer to make up volume to 26ul, 4ul of lOx reducing agent and

lOul 4x SDS loading buffer (both from Life Technologies, Grand Island, NY). Samples were heated at 95oC for 5min and loaded on the gel. Standard settings were chosen by the manufacturer, 200V, 120mA and max. 25W. Run time was 60min, but no longer than running dye reaching the lower end of the gel.

[001066] After the run was terminated, the plastic case was cracked and the encased gel transferred to a ready-to-use nitrocellulose membrane kit and power supply (iBLOT; LifeTechnologies, Grand Island, NY). Using default settings, the protein lysate was transferred by high Ampere electricity from the gel to the membrane.

[001067] After the transfer, the membranes were incubated in 5% BSA in IX TBS for 15 minutes then in 5% BSA in IX TBS + 0.1 % Tween for another 15 minutes. Primary antibodies (Granulysin mouse monoclonal antibody; Abeam, Cambridge, MA) against granulysin proteins were applied in 3ml of 5% BSA in IX TBS solution at a 1:500 to 1:2000 dilution for 3 hours at room temperature and gentle agitation on an orbital shaker. Membranes are washed 3 times with IX TBS/0.1 % Tween, 5 minutes each time with gentle agitation. The secondary antibody (Donkey anti-mouse HRP conjugate; Abeam, Cambridge, MA) was conjugated to horse radish peroxidase and binds to the primary antibody antibodies. The secondary antibody was diluted of 1:1000 to 1:5000 in 5% BSA in IX TBS and incubated for 3 hrs at RT.

[001068] At the end of incubation time, the membranes were washed 3 times with IX TBS/0.1 % Tween, 5 minutes each time with gentle agitation. The membranes were developed in 5ml Pierce WestPico Chemiluminescent Subtrate (Thermo Fisher, Rockford, IL) as directed.

[001069] As shown in Figures 6A and 6B the Western Blot detected protein around the expected size of 16 kd for each of the 2 samples evaluated for each chemistry.

Example 31. Confirmation of Peptide Identity

[001070] Proteins can be evaluated using liquid chromatography-mass spectrometry in tandem with mass spectrometry (LC-MS/MS) with quantitative LC-multiple reaction monitoring (MRM) in order to confirm the identity of the peptide.

The identity of any protein target described herein can be evaluated using the liquid chromatography-mass spectrometry in tandem with mass spectrometry (LC-MS/MS) with quantitative LC-multiple reaction monitoring (MRM) Assay (Biognosys AG,

Schlieren Switzerland). HeLa cell lysates containing protein expressed from modified mRNA are evaluated using LC-MS/MS with quantitative LC-MRM Assay (Biognosys, Schlieren Switzerland) in order to confirm the identity of the peptides in the cell lysates. The identified peptide fragments are compared against known proteins including isoforms using methods known and/or described in the art.

A. Sample Preparation

[001071] Protein in each sample in lysis buffer is reduced by incubation for 1 hour at 37°C with 5 mM tris(2-carboxyethyl)phosphine (TCEP). Alkylation is carried out using 10 mM iodoacetamide for 30 minutes in the dark at room temperature. Proteins are digested to peptides using trypsin (sequence grade, PromegaCorporation, Madison, WI) at a protease: protein ratio of 1:50. Digestion is carried out overnight at 37°C (total digestion time is 12 hours). Peptides are cleaned up for mass spectrometric analysis using C18 spin columns (The Nest Group, Southborough, MA) according to the manufacturer's instructions. Peptides are dried down to complete dryness and resuspended in LC solvent A (1% acetonitrile, 0.1% formic acid (FA)). All solvents are HPLC-grade from SIGMA-ALDRICH® (St. Louis, MO) and all chemicals, where not stated otherwise, are obtained from SIGMA-ALDRICH® (St. Louis, MO).

B. LC-MS/MS and LC-MRM

[001072] Peptides are injected to a packed C18 column (Magic AQ, 3 um particle size, 200 **A** pore size, Michrom Bioresources, Inc (Auburn, CA); 11 cm column length, 75 um inner diameter, New Objective (Woburn, MA)) on a Proxeon Easy nLC nano-liquid chromatography system for all mass spectrometric analysis. LC solvents are A: 1 % acetonitrile in water with 0.1% FA; B: 3% water in acetonitrile with 0.1% FA. The LC gradient for shotgun analysis is 5-35% solvent B in 120 minutes followed by 35-100 % solvent B in 2 minutes and 100 % solvent B for 8 minutes (total gradient length is 130 minutes). LC-MS/MS shotgun runs for peptide discovery are carried out on a Thermo Scientific (Thermo Fisher Scientific) (Billerica, MA) Q Exactive mass spectrometer equipped with a standard nano-electrospray source. The LC gradient for LC-MRM is 5-35% solvent B in 30 minutes followed by 35-100% solvent B in 2 minutes and *100%* solvent B for 8 minutes (total gradient length is 40 minutes). The Thermo Scientific (Thermo Fisher Scientific) (Billerica, MA) TSQ Vantage triple quadrupole mass spectrometer is equipped with a standard nano-electrospray source. In unscheduled

MRM mode for recalibration it is operated at a dwell time of 20 ms per transition. For relative quantification of the peptides across samples, the TSQ Vantage is operated in scheduled MRM mode with an acquisition window length of 4 minutes. The LC eluent is electrosprayed at 1.9 kV and MRM analysis is performed using a Q1 peak width of 0.7 Da. Collision energies are calculated for the TSQ Vantage by a linear regression according to the vendor's specifications.

C. Assay Design, Data Processing and Analysis

[001073] For the generation of LC-MRM assays, the 12 most intense fragment ions from LC-MS/MS analysis are measured in scheduled LC-MRM mode and data were processed using MQUEST® (Cluetec, Karlsruhe, Germany), the scoring part of mProphet (Reiter et al, mProphet: Automated data processing and statistical validation for large-scale SRM experiments, Nature Methods, 201 1 (8), 430-435; the contents of which are herein incorporated by reference). Assays were validated manually, exact fragment intensities are determined and iRTs (indexed retention times) are assigned relative to Biognosys's iRT-peptides (Escher et al. Using iRT, a normalized retention time for more targeted measurement of peptides, Proteomics, 2012 (12), 1111-1 121; the contents of which are herein incorporated by reference).

For the relative quantification of the peptides across the sample series the 8 most intense transitions of each assay are measured across the sample series. Data analysis is carried out using SpectroDive™ (Biognosys, Schlieren Switzerland). Total peak areas are compared for the selected peptides and a false discover rate of 0.05 is applied. Peptides with a Qvalue below 0.05 are excluded and considered not detected in the respective sample.

Example 32. Confirmation and of Peptide Identity from Chemically Modified mRNA

[001074] Cell lysates containing protein produced from siah E3 ubiquitin protein ligase 1 (SIAH1) modified mRNA (mRNA sequence shown in SEQ ID NO. 6618 (Table 29); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), MYC inhibitor D (a unique dominant-negative 90 amino acid protein comprised of the human c-Myc) modified mRNA (mRNA sequence shown in SEQ ID NO. 6621 (Table 29); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), fully

modified with 5-methylcytidine and pseudouridine (5mC and pU), fully modified with 5-methylcytidine and 1-methylpsudouridine (5mC and ImpU), modified where 25% of uridine modified with 2-thiouridine and 25% of cytidine modified with 5-methylcytidine (s2U and 5mC), fully modified with pseudouridine (pU), or fully modified with 1-methylpseudouridine (ImpU) were evaluated using the LC-MS/MS with quantitative LC-MRM as described in Example 31. Peptide fragments identified for the evaluated proteins are shown in Table 30.

Table 30. Proteins and Peptide Fragment Sequences

	Peptide Fragment SEQ ID NO	5mC and pU	5mC and 1mpU	s2U and 5mC	pU	1mpU
SIAH1						
GPLGSIR	6622	YES	-	YES	-	YES
MYC INHIBITOR D						
ATAYILSVQAETQK	6623	YES	YES	YES	YES	YES
KATAYILSVQAETQK	6624	YES	YES	YES	YES	YES
LISEIDLLRK	6625	YES	YES	YES	YES	YES

Example 33. Confirmation and of Peptide Identity from 1-methylpseudouridine Modified mRNA

[001075] Cell lysates containing protein produced from granulysin mRNA (mRNA sequence shown in Table 29; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) fully modified with 1-methylpseudouridine (ImpU) were evaluated using the LC-MS/MS with quantitative LC-MRM as described in Example 31. Peptide fragments identified for the evaluated proteins are shown in Table 31. In Table 31, "Uniprot ID" refers to the protein identifier from the UniProt database when the peptide fragment sequences were blasted against all review proteins in the database.

Table 31. Proteins and Peptide Fragment Sequences

	Peptide Fragment SEQ ID NO	Uniprot ID
GRANULYSIN		
SCPCLAQEGPQGDLLTK	6626	P22749

Example 34. Signal-sensor Polynucleotides in the treatment of cancer (HCC): disruption of cancer cell transcriptome using dominant negative STAT3 and Akt mRNA.

[001076] Using the animal models outlined in Example 13, animals are treated with signal-sensor polynucleotide encoding for a dominant negative STAT3 molecule or a dominant negative Akt molecule whose expression has been shown to interfere with PI-3 kinase induced oncogenic transformation, including in glioblastoma cells (Vogt and Hart, Cancer Discov, 201 1 1:48 1-486; herein included by reference in its entirety). Animals are injected with mRNA encoding dominant negative STAT3 mRNA vs dominant negative Akt mRNA vs negative control mRNA (non-translated version of the same mRNA containing multiple stop codons) vs vehicle using an appropriate route of delivery and formulation. Animals are then evaluated for gene expression, tumor status or for any of the hallmarks associated with cancer phenotypes or genotypes. Other examples of dominant negative approaches for cancer are outlined and could similarly be used with modified mRNA (Moss and Lemoine Chapter 15 RNA Interference and Dominant Negative Approaches in Viral Therapy of Cancer Harrington et al., eds. Wiley & Sons; herein incorporated by reference in its entirety).

Example 35. Signal-sensor Polynucleotides in the treatment of cancer (HCC): disruption of cancer cell transcriptome using dominant negative hTERT mRNA.

[001077] Using the animal models outlined in Example 13, animals are treated with signal-sensor polynucleotide encoding for a dominant negative hTERT whose expression has been shown to interfere with telomerase activity and lead to apoptosis of cancer cells (Agrawal et al. 2012 Recent Pat Anricancer Drug Discov 7:102-1 17, Samy et al. 2012 Mol Cancer Ther 11:2384-2393, Nguyen et al. 2009 Cell Cycle. 8:3227-3233; all herein included by reference in their entirety). Telomerase, a specialised RNA-directed DNA polymerase extends and stabilises the telomeres at the ends of the eukaryotic chromosomes. The progressive loss of telomeres results in limited number of cell divisions and has been linked to the mechanism of human cellular ageing. Tumour cells marked by indefinite proliferation have stable telomere length maintained by telomerase. The differential expression of the telomerase enzyme in normal and cancer cells have led to the evolution of tumour specific anti-telomerase approaches which inhibit the

telomerase enzyme activity so as to destabilise and shorten the telomeres leading to senescence in cancer cells. One such approach is to use modified mRNA to express a dominant negative hTERT. As such animals are injected with mRNA encoding dominant negative hTERT mRNA vs negative control mRNA (non-translated version of the same mRNA containing multiple stop codons) vs vehicle using an appropriate route of delivery and formulation. Animals are then evaluated for gene expression, tumor status or for any of the hallmarks associated with cancer phenotypes or genotypes. Other examples of dominant negative approaches for cancer are outlined and could similarly be used with modified mRNA (Moss and Lemoine Chapter 15 RNA Interference and Dominant Negative Approaches in Viral Therapy of Cancer Harrington et al., eds. Wiley & Sons; herein incorporated by reference in its entirety).

Example 36. Signal-sensor Polynucleotides in the treatment of cancer (HCC): disruption of cancer cell transcriptome using dominant negative survivin mRNA.

[001078] Using the animal models outlined in Example 13, animals are treated with signal-sensor polynucleotide encoding for a dominant negative survivin (C84A and others) whose expression has been shown to lead to apoptosis of cancer cells (Cheung et al. 2010 Cancer Cell Int. 10:36; herein included by reference in its entirety). Survivin is a member of the inhibitor-of-apoptosis (IAP) family which is widely expressed by many different cancers. Overexpression of survivin is associated with drug resistance in cancer cells, and reduced patient survival after chemotherapy and radiotherapy. Agents that antagonize the function of survivin hold promise for treating many forms of cancer. One such approach is to use modified mRNA to express a dominant negative survivin (C84A mutation is one described example). As such animals are injected with mRNA encoding dominant negative survivin mRNA vs negative control mRNA (non-translated version of the same mRNA containing multiple stop codons) vs vehicle using an appropriate route of delivery and formulation. Animals are then evaluated for gene expression, tumor status or for any of the hallmarks associated with cancer phenotypes or genotypes. Other examples of dominant negative approaches for cancer are outlined and could similarly be used with modified mRNA (Moss and Lemoine Chapter 15_RNA Interference and Dominant Negative Approaches in Viral Therapy of Cancer Harrington et al., eds. Wiley & Sons; herein incorporated by reference in its entirety).

Example 37. Expression of modified nucleic acid with microRNA binding site

[001079] Human embryonic kidney epithelial cells (HEK293A), or antigen presenting cells or cell lines with highly expressed mir-142/146, such as monocyte-derived dendritic cells (MDDC) or PBMC, are seeded at a density of 200,000 per well in 500 ul cell culture medium (InVitro GRO medium from Celsis, Chicago, IL). G-CSF mRNA (mRNA sequence is shown in SEQ ID NO: 6595; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a miR-142-5p binding site (G-CSF miR-142-5p) (cDNA sequence is shown in SEQ ID NO:6627; mRNA sequence is shown in SEQ ID NO: 6628, polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl), G-CSF mRNA having a seed sequence from miR-142-5p binding site (G-CSF miR-142-5p-seed) (cDNA sequence is shown in SEQ ID NO. 6629; mRNA sequence is shown in SEQ ID NO: 6630; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a miR-142-5p binding site without the seed sequence (G-CSF miR-142-5p-seedless) (cDNA sequence is shown in SEQ ID NO: 6631, mRNA sequence is shown in SEQ ID NO: 6632; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a miR-142-3p binding site (G-CSF miR-142-3p) (cDNA sequence is shown in SEQ ID NO: 6633, mRNA sequence is shown in SEQ ID NO: 6634; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl; G-CSF mRNA having a seed sequence from miR-142-3p binding site (G-CSF miR-142-3p-seed) (cDNA sequence is shown in SEQ ID NO: 6635, mRNA sequence is shown in SEQ ID NO: 6636; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a miR-142-3p binding site without the seed sequence (G-CSF miR-142-3p-seedless) (cDNA sequence is shown in SEQ ID NO: 6637; mRNA sequence is shown in SEQ ID NO: 6638; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a miR-146a binding site (G-CSF miR-146a) (cDNA sequence is shown in SEQ ID NO. 6639, mRNA sequence is shown in SEQ ID NO: 6640; polyA tail of at least 140 nucleotides not shown in sequence; 5'Cap, Capl) G-CSF mRNA having a seed sequence from miR-146a binding site (G-CSF miR-146a-seed) (cDNA sequence is shown in SEQ ID N0.6641, mRNA sequence is shown in SEQ ID NO:6642; polyA tail at least 140 nucleotides not shown in sequence; 5'Cap,Capl) or G-CSF mRNA having a miR-146a

binding site without the seed sequence(G-CSF miR-146a-seedless) (cDNA sequence is shown in SEQ ID N0.6643, mRNA sequence is shown in SEQ ID NO: 6644; polyA tail at least nucleotides not shown in sequence; 5'Cap, Capl) are tested at a concentration of 250 ng per well in 24 well plates. The mRNA sequences are evaluated with various chemical modifications described herein and/or known in the art including, fully modified with 5-methylcytidine and pseudouridine, fully modified with 5-methylcytidine and 1-methylpseudouridine, fully modified with pseudouridine, fully modified with 1-methylpseudouridine and where 25% of the uridine residues are modified with 2-thiouridine and 25% of the cytidine residues are modified with 5-methylcytidine. The expression of G-CSF in each sample is measured by ELISA.

[001080] Shown in Table 32 are the DNA and mRNA G-CSF seuqences with the miR binding sites described above. In the table, the start codon of each sequence is underlined.

Table 32. G-CSF constructs with miR binding sites

SEQ ID NO.	Description	SEQ
6627	DNA sequence having the T7 polymerase site and restriction sites: G-CSF miR-142-5p	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCC ACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGCC CTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCAAGAAG CGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCATTCCTTTTG AAGTGTCTGGAGCAGGTGCGAAAGATTCAGGGCGATGGAGCCGCA CTCCAAGAGAAGCTCTGCGCGACATACAAACTTTGCCATCCCGAG GAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCCTGGGCTCCTC TCTCGTCCTGTCCGTCGCAGGCTTTGCAGTTGGCAGGGTGCCTTTC CCAGCTCCACTCCGGTTTGTTCTTGTATCAGGGACTGCTGCAAGCC CTTGAGGGAATCTCGCCAGAATTGGGCCCGACGCTGGACACGTTG CAGCTCGACGTGGCGGATTTCGCAACAACCATCTGGCAGCAGATG GAGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGGC AATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGTGGAGT CCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTCTCGTACCGG GTGCTGAGACATCTTGCGCAGCCGTGATAATAGGCTGCCTTCTGCG GGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTAC CTCTAGTAGTGCTTTCTACTTTATGTGGTCTTTGAATAAAGCCTGA GTAGGAAGGCGGCCGCTCGAGCATGCATCTAGA
6628	mRNA sequence: G-CSF miR-142-5p	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGC CACCAUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAA GAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCAUU CCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGCGAU GGAGCCGCACUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUG

		CCAUCCCGAGGAGCUCGUACUGCUCGGGCACAGCUUGGGGAUUC CCUGGGCUCCUCUCUCGUCCUGUCCGUCGCAGGCUUUGCAGUUG GCAGGGUGCCUUUCCCAGCUCCACUCCGGUUUGUUCUUGUAUCA GGGACUGCUGCAAGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCC CGACGCUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACA ACCAUCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCGUUUC AGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACCUUCAAUCA UUUUUGGAAGUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCC GUGAUAAUAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGC CCUUCUUCUCUCCCUUGCACCUGUACCUCUAGUAGUGCUUUCUA CUUUAUGUGGUCUUUGAAUAAAGCCUGAGUAGGAAG
6629	DNA sequence having the T7 polymerase site and restriction sites: G-CSF miR-142-5p-seed	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCC ACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGCC CTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCAAGAAG CGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCATTCCTTTTG AAGTGTCTGGAGCAGGTGCGAAAGATTCAGGGCGATGGAGCCGCA CTCCAAGAGAAGCTCTGCGCGACATACAAACTTTGCCATCCCGAG GAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCCTGGGCTCCTC TCTCGTCCTGTCCGTCGCAGGCTTTGCAGTTGGCAGGGTGCCTTTC CCAGCTCCACTCCGGTTTGTTCTTGTATCAGGGACTGCTGCAAGCC CTTGAGGGAATCTCGCCAGAATTGGGCCCGACGCTGGACACGTTG CAGCTCGACGTGGCGGATTTCGCAACAACCATCTGGCAGCAGATG GAGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGGC AATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGTGGAGT CCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTCTCGTACCGG GTGCTGAGACATCTTGCGCAGCCGTGATAATAGGCTGCCTTCTGCG GGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTAC CTCTACTTTATTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGC CGCTCGAGCATGCATCTAGA
6630	mRNA sequence: G-CSF miR-142-5p-seed	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGC CACC AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGGCCCU GCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAAGAAG CGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCAUUCCUU UUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGCGAUGGAG CCGCACUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUGCCAU CCCGAGGAGCUCGUACUGCUCGGGCACAGCUUGGGGAUUCCCUG GGCUCCUCUCUCGUCCUGUCCGUCGCAGGCUUUGCAGUUGGCAG GGUGCCUUUCCCAGCUCCACUCCGGUUUGUUCUUGUAUCAGGGA CUGCUGCAAGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGAC GCUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCUGCAG CCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCGUUUCAGCG CAGGGCGGGUGGAGUCCUCGUAGCGAGCCACCUUCAAUCAUUUU UGGAAGUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCCG UGAUAAUAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCC CUUCUUCUCUCCCUUGCACCUGUACCUCUACUUUAUUGGUCUUU GAAUAAAGCCUGAGUAGGAAG
6631	DNA sequence having the T7 polymerase	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCC ACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGCC CTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCAAGAAG CGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCATTCCTTTTG

	site and restriction sites: G-CSF miR-142-5p-seedless	AAGTGTCTGGAGCAGGTGCGAAAGATTCAGGGCGATGGAGCCGCA CTCCAAGAGAAGCTCTGCGCGACATACAAACTTTGCCATCCCGAG GAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCCTGGGCTCCTC TCTCGTCCTGTCCGTCGCAGGCTTTGCAGTTGGCAGGGTGCCTTTC CCAGCTCCACTCCGGTTTGTTCTTGTATCAGGGACTGCTGCAAGCC CTTGAGGGAATCTCGCCAGAATTGGGCCCGACGCTGGACACGTTG CAGCTCGACGTGGCGGATTTCGCAACAACCATCTGGCAGCAGATG GAGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGGC AATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGTGGAGT CCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTCTCGTACCGG GTGCTGAGACATCTTGCGCAGCCGTGATAATAGGCTGCCTTCTGCG GGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTAC CTCTAGTAGTGCTTTCTGTGGTCTTTGAATAAAGCCTGAGTAGGAA GGCGGCCGCTCGAGCATGCATCTAGA
6632	mRNA sequence: G-CSF miR-142-5p-seedless	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGC CACC AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGGCCCU GCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAAGAAG CGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCAUUCCUU UUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGCGAUGGAG CCGCACUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUGCCAU CCCGAGGAGCUCGUACUGCUCGGGCACAGCUUGGGGAUUCCCUG GGCUCCUCUCUCGUCCUGUCCGUCGCAGGCUUUGCAGUUGGCAG GGUGCCUUUCCCAGCUCCACUCCGGUUUGUUCUUGUAUCAGGGA CUGCUGCAAGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGAC GCUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCUGCAG CCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCGUUUCAGCG CAGGGCGGGUGGAGUCCUCGUAGCGAGCCACCUUCAAUCAUUUU UGGAAGUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCCG UGAUAAUAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCC CUUCUUCUCUCCCUUGCACCUGUACCUCUAGUAGUGCUUUCUGU GGUCUUUGAAUAAAGCCUGAGUAGGAAG
6633	DNA sequence having the T7 polymerase site and restriction sites: G-CSF miR-142-3p	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCC ACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGCC CTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCAAGAAG CGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCATTCCTTTTG AAGTGTCTGGAGCAGGTGCGAAAGATTCAGGGCGATGGAGCCGCA CTCCAAGAGAAGCTCTGCGCGACATACAAACTTTGCCATCCCGAG GAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCCTGGGCTCCTC TCTCGTCCTGTCCGTCGCAGGCTTTGCAGTTGGCAGGGTGCCTTTC CCAGCTCCACTCCGGTTTGTTCTTGTATCAGGGACTGCTGCAAGCC CTTGAGGGAATCTCGCCAGAATTGGGCCCGACGCTGGACACGTTG CAGCTCGACGTGGCGGATTTCGCAACAACCATCTGGCAGCAGATG GAGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGGC AATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGTGGAGT CCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTCTCGTACCGG GTGCTGAGACATCTTGCGCAGCCGTGATAATAGGCTGCCTTCTGCG GGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTAC CTCTTCCATAAAGTAGGAAACACTACATGGTCTTTGAATAAAGCCT GAGTAGGAAGGCGGCCGCTCGAGCATGCATCTAGA
6634	mRNA sequence: G-CSF miR-	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGC CACC AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGGCCCU

	142-3p	GCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAAGAAG CGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCAUUCCUU UUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGCGAUGGAG CCGCACUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUGCCAU CCCGAGGAGCUCGUACUGCUCGGGCACAGCUUGGGGAUUCCCUG GGCUCCUCUCUCGUCCUGUCCGUCGCAGGCUUUGCAGUUGGCAG GGUGCCUUUCCCAGCUCCACUCCGGUUUGUUCUUGUAUCAGGGA CUGCUGCAAGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGAC GCUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCUGCAG CCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCGUUUCAGCG CAGGGCGGGUGGAGUCCUCGUAGCGAGCCACCUUCAAUCAUUUU UGGAAGUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCCG UGAUAAUAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCC CUUCUUCUCUCCCUUGCACCUGUACCUCUUCCAUAAAGUAGGAA ACACUACAUGGUCUUUGAAUAAAGCCUGAGUAGGAAG
6635	DNA sequence having the T7 polymerase site and restriction sites: G-CSF miR-142-3p-seed	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCC ACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGCC CTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCAAGAAG CGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCATTCCTTTTG AAGTGTCTGGAGCAGGTGCGAAAGATTCAGGGCGATGGAGCCGCA CTCCAAGAGAAGCTCTGCGCGACATACAAACTTTGCCATCCCGAG GAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCCTGGGCTCCTC TCTCGTCCTGTCCGTCGCAGGCTTTGCAGTTGGCAGGGTGCCTTTC CCAGCTCCACTCCGGTTTGTTCTTGTATCAGGGACTGCTGCAAGCC CTTGAGGGAATCTCGCCAGAATTGGGCCCGACGCTGGACACGTTG CAGCTCGACGTGGCGGATTTCGCAACAACCATCTGGCAGCAGATG GAGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGGC AATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGTGGAGT CCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTCTCGTACCGG GTGCTGAGACATCTTGCGCAGCCGTGATAATAGGCTGCCTTCTGCG GGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTAC CTCTACACTACTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGC CGCTCGAGCATGCATCTAGA
6636	mRNA sequence: G-CSF miR-142-3p-seed	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGC CACC AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGGCCCU GCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAAGAAG CGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCAUUCCUU UUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGCGAUGGAG CCGCACUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUGCCAU CCCGAGGAGCUCGUACUGCUCGGGCACAGCUUGGGGAUUCCCUG GGCUCCUCUCUCGUCCUGUCCGUCGCAGGCUUUGCAGUUGGCAG GGUGCCUUUCCCAGCUCCACUCCGGUUUGUUCUUGUAUCAGGGA CUGCUGCAAGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGAC GCUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACAACCA UCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCUGCAG CCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCGUUUCAGCG CAGGGCGGGUGGAGUCCUCGUAGCGAGCCACCUUCAAUCAUUUU UGGAAGUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCCG UGAUAAUAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCC CUUCUUCUCUCCCUUGCACCUGUACCUCUACACUACUGGUCUUU GAAUAAAGCCUGAGUAGGAAG
6637	DNA	TAATACGACTCACTATA

	sequence having the T7 polymerase site and restriction sites: G-CSF miR-142-3p-seedless	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCC ACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGCC CTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCAAGAAG CGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCATTCCTTTTG AAGTGTCTGGAGCAGGTGCGAAAGATTCAGGGCGATGGAGCCGCA CTCCAAGAGAAGCTCTGCGCGACATACAAACTTTGCCATCCCGAG GAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCCTGGGCTCCTC TCTCGTCCTGTCCGTCGCAGGCTTTGCAGTTGGCAGGGTGCCTTTC CCAGCTCCACTCCGGTTTGTTCTTGTATCAGGGACTGCTGCAAGCC CTTGAGGGAATCTCGCCAGAATTGGGCCCGACGCTGGACACGTTG CAGCTCGACGTGGCGGATTTCGCAACAACCATCTGGCAGCAGATG GAGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGGC AATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGTGGAGT CCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTCTCGTACCGG GTGCTGAGACATCTTGCGCAGCCGTGATAATAGGCTGCCTTCTGCG GGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTAC CTCTTCCATAAAGTAGGAAATGGTCTTTGAATAAAGCCTGAGTAG GAAGGCGGCCGCTCGAGCATGCATCTAGA
6638	mRNA sequence: G-CSF miR-142-3p-seedless	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGC CACCAUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAA GAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCAUU CCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGCGAU GGAGCCGCACUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUG CCAUCCCGAGGAGCUCGUACUGCUCGGGCACAGCUUGGGGAUUC CCUGGGCUCCUCUCUCGUCCUGUCCGUCGCAGGCUUUGCAGUUG GCAGGGUGCCUUUCCCAGCUCCACUCCGGUUUGUUCUUGUAUCA GGGACUGCUGCAAGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCC CGACGCUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACA ACCAUCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCGUUUC AGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACCUUCAAUCA UUUUUGGAAGUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCC GUGAUAAUAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGC CCUUCUUCUCUCCCUUGCACCUGUACCUCUUCCAUAAAGUAGGA AAUGGUCUUUGAAUAAAGCCUGAGUAGGAAG
6639	DNA sequence having the T7 polymerase site and restriction sites: G-CSF miR-146a	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCC ACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGCC CTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCAAGAAG CGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCATTCCTTTTG AAGTGTCTGGAGCAGGTGCGAAAGATTCAGGGCGATGGAGCCGCA CTCCAAGAGAAGCTCTGCGCGACATACAAACTTTGCCATCCCGAG GAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCCTGGGCTCCTC TCTCGTCCTGTCCGTCGCAGGCTTTGCAGTTGGCAGGGTGCCTTTC CCAGCTCCACTCCGGTTTGTTCTTGTATCAGGGACTGCTGCAAGCC CTTGAGGGAATCTCGCCAGAATTGGGCCCGACGCTGGACACGTTG CAGCTCGACGTGGCGGATTTCGCAACAACCATCTGGCAGCAGATG GAGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGGC AATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGTGGAGT CCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTCTCGTACCGG GTGCTGAGACATCTTGCGCAGCCGTGATAATAGGCTGCCTTCTGCG GGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTAC CTCTAACCCATGGAATTCAGTTCTCATGGTCTTTGAATAAAGCCTG AGTAGGAAGGCGGCCGCTCGAGCATGCATCTAGA

6640	mRNA sequence: G-CSF miR-146a	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGC CACCAUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAA GAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCAUU CCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGCGAU GGAGCCGCACUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUG CCAUCCCGAGGAGCUCGUACUGCUCGGGCACAGCUUGGGGAUUC CCUGGGCUCCUCUCUCGUCCUGUCCGUCGCAGGCUUUGCAGUUG GCAGGGUGCCUUUCCCAGCUCCACUCCGGUUUGUUCUUGUAUCA GGGACUGCUGCAAGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCC CGACGCUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACA ACCAUCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCGUUUC AGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACCUUCAAUCA UUUUUGGAAGUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCC GUGAUAAUAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGC CCUUCUUCUCUCCCUUGCACCUGUACCUCUAACCCAUGGAAUUC AGUUCUCAUGGUCUUUGAAUAAAGCCUGAGUAGGAAG
6641	DNA sequence having the T7 polymerase site and restriction sites: G-CSF-146a-seed	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCC ACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGCC CTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCAAGAAG CGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCATTCCTTTTG AAGTGTCTGGAGCAGGTGCGAAAGATTCAGGGCGATGGAGCCGCA CTCCAAGAGAAGCTCTGCGCGACATACAAACTTTGCCATCCCGAG GAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCCTGGGCTCCTC TCTCGTCCTGTCCGTCGCAGGCTTTGCAGTTGGCAGGGTGCCTTTC CCAGCTCCACTCCGGTTTGTTCTTGTATCAGGGACTGCTGCAAGCC CTTGAGGGAATCTCGCCAGAATTGGGCCCGACGCTGGACACGTTG CAGCTCGACGTGGCGGATTTCGCAACAACCATCTGGCAGCAGATG GAGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGGC AATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGTGGAGT CCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTCTCGTACCGG GTGCTGAGACATCTTGCGCAGCCGTGATAATAGGCTGCCTTCTGCG GGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTAC CTCTAGTTCTCTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGC CGCTCGAGCATGCATCTAGA
6642	mRNA sequence: G-CSF-146a-seed	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGC CACCAUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAA GAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCAUU CCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGCGAU GGAGCCGCACUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUG CCAUCCCGAGGAGCUCGUACUGCUCGGGCACAGCUUGGGGAUUC CCUGGGCUCCUCUCUCGUCCUGUCCGUCGCAGGCUUUGCAGUUG GCAGGGUGCCUUUCCCAGCUCCACUCCGGUUUGUUCUUGUAUCA GGGACUGCUGCAAGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCC CGACGCUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACA ACCAUCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCGUUUC AGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACCUUCAAUCA UUUUUGGAAGUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCC GUGAUAAUAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGC CCUUCUUCUCUCCCUUGCACCUGUACCUCUAGUUCUCUGGUCUU UGAAUAAAGCCUGAGUAGGAAG

6643	DNA sequence having the T7 polymerase site and restriction sites: G-CSF-146a-seedless	TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCC ACCATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGCC CTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGGACAGTCCAAGAAG CGACTCCTCTCGGACCTGCCTCATCGTTGCCGCAGTCATTCCTTTTG AAGTGTCTGGAGCAGGTGCGAAAGATTCAGGGCGATGGAGCCGCA CTCCAAGAGAAGCTCTGCGCGACATACAAACTTTGCCATCCCGAG GAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCCTGGGCTCCTC TCTCGTCCTGTCCGTCGCAGGCTTTGCAGTTGGCAGGGTGCCTTTC CCAGCTCCACTCCGGTTTGTTCTTGTATCAGGGACTGCTGCAAGCC CTTGAGGGAATCTCGCCAGAATTGGGCCCGACGCTGGACACGTTG CAGCTCGACGTGGCGGATTTCGCAACAACCATCTGGCAGCAGATG GAGGAACTGGGGATGGCACCCGCGCTGCAGCCCACGCAGGGGGC AATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCGGGTGGAGT CCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTCTCGTACCGG GTGCTGAGACATCTTGCGCAGCCGTGATAATAGGCTGCCTTCTGCG GGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTAC CTCTAACCCATGGAATTCATGGTCTTTGAATAAAGCCTGAGTAGGA AGGCGGCCGCTCGAGCATGCATCTAGA
6644	mRNA sequence: G-CSF-146a-seedless	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGC CACCAUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGG CCCUGCAGUUGCUGCUUUGGCACUCGGCCCUCUGGACAGUCCAA GAAGCGACUCCUCUCGGACCUGCCUCAUCGUUGCCGCAGUCAUU CCUUUUGAAGUGUCUGGAGCAGGUGCGAAAGAUUCAGGGCGAU GGAGCCGCACUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUG CCAUCCCGAGGAGCUCGUACUGCUCGGGCACAGCUUGGGGAUUC CCUGGGCUCCUCUCUCGUCCUGUCCGUCGCAGGCUUUGCAGUUG GCAGGGUGCCUUUCCCAGCUCCACUCCGGUUUGUUCUUGUAUCA GGGACUGCUGCAAGCCCUUGAGGGAAUCUCGCCAGAAUUGGGCC CGACGCUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCAACA ACCAUCUGGCAGCAGAUGGAGGAACUGGGGAUGGCACCCGCGCU GCAGCCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCGCGUUUC AGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACCUUCAAUCA UUUUUGGAAGUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCC GUGAUAAUAGGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGC CCUUCUUCUCUCCCUUGCACCUGUACCUCUAACCCAUGGAAUUC AUGGUCUUUGAAUAAAGCCUGAGUAGGAAG

[001081] It is likely that the binding site "seed" sequence is sufficient to induce mircoRNA binding, the expression of G-CSF should be down-regulated in cells transfected with miR-142-3p, miR-142-3p-seed, miR-142-5p, miR-142-5p-seed, miR-146a or miR-146a-seed. Whereas, the miR-142-3p-seedless, miR-142-5p-seedless, miR-146a-seedless should not change the expression of G-CSF, as compared with cells transfected with G-CSF mRNA without microRNA binding sites.

Example 38. APCs specific microRNA binding sites to suppress modified nucleic acid mediated immune stimulation

[001082] The binding sites for microRNAs are used in the 3'UTR of mRNA therapeutics to selectively degrade mRNA therapeutics in the immune cells to subdue unwanted immunogenic reactions caused by mRNA therapeutics delivery.

[001083] A signal-sensor polynucleotide comprising a series of 3'UTR miR binding sites which make the signal sensor polynucleotide more unstable in antigen presenting cells (APCs), such as, but not limited to mir-142-5p, mir-142-3p, mir-146a-5p and mir-146a-3p, encodes an oncology-related polypeptide of the present invention. The addition of miR binding sites in the 3'UTR making a signal sensnor polynucleotide unstable would subdue modified mRNA mediated immune stimulation.

[001084] Experiments comparing the cytokine expression (e.g. TNF-alpha) induced by the signal-sensor polypeptide with APCs specific microRNA signature vs. without such signature is performed in vitro by methods described herein and/or known in the art.

Example 39. *In Vitro* Expression of mRNAs with miR binding sites

[001085] Human embryonic kidney epithelial cells (HEK293A), antigen-presenting cells or cell lines with highly expressed mir-142/146, such as monocyte-derived dendritic cells (MDDC) or PBMC, are seeded at a density of 200,000 per well in 500 ul cell culture medium (InVitro GRO medium from Celsis, Chicago, IL). Cultured cells are transfected with G-CSF mRNAs with or without microRNA signature, as described in Example 37. The cells are transfected for five consecutive days. The transfection complexes are removed four hours after each round of transfection.

[001086] The culture supernatant is assayed for secreted G-CSF (R&D Systems, catalog #DCS50), tumor necrosis factor-alpha (TNF-alpha) and interferon alpha (IFN-alpha by ELISA every day after transfection following manufacturer's protocols. The cells are analyzed for viability using CELL TITER GLO® (Promega, catalog #G7570) 6 hrs and 18 hrs after the first round of transfection and every alternate day following that. At the same time from the harvested cells, total RNA is isolated and treated with DNASE® using the RNAEASY micro kit (catalog #74004) following the manufacturer's protocol. 100 ng of total RNA is used for cDNA synthesis using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, cat #4368814) following the manufacturer's protocol. The cDNA is then analyzed for the expression of innate immune response

genes by quantitative real time PCR using SybrGreen in a Biorad CFX 384 instrument following the manufacturer's protocol.

Example 40. In vivo detection of innate immune response study

[001087] To test the signal sensor protein expression and in vivo immune response, female BALB/C mice (n=5) are injected intramuscularly with G-CSF mRNA with or without microRNA signatures as described in Example 37. Blood is collected at 8 hours after dosing. The protein levels of G-CSF, TNF-alpha and IFN-alpha is determined by ELISA.

[001088] The difference of cytokine production is seen as measured by mouse TNF-alpha and IFN-alpha level in serum. Injection with G-CSF modified mRNA having miR-142 and miR-146a binding site or binding site seed shows a lower level of cytokine response in vivo.

Example 41. Expression of miR-122 in primary hepatocytes

[001089] Hepatocyte specific miR-122 level in rat and human primary hepatocytes was measured. Hela Cells and primary rat and human hepatocytes were cultured and RNAs were extracted from cell lysates. The miR-122 level in rat and human primary hepatocytes was compared with that in Hela cells. The miR-122 level is about 6 fold increased in primary human hepatocytes and about 12 fold increased in primary rat hepatocytes, respectively, as compared with that in Hela cells.

Example 42. Expression of modified nucleic acid with mir-122 binding site in hepatocytes

[001090] Primary rat and human hepatocytes and Hela cells were seeded at a density of 200,000 per well in 500 ul cell culture medium (InVitro GRO medium from Celsis, Chicago, IL). G-CSF mRNA having a miR-122 binding site in the 3'UTR (G-CSF miR-122-1X) (mRNA sequence is shown in SEQ ID NO: 6600; polyA tail of approximately 140 nucleotides not shown in sequence; 5'Cap, Capl) fully modified with 5-methylcytidine and pseudouridine (5mC/pU), or fully modified with pseudouridine(pU) or G-CSF mRNA with four miR-122 binding sites with the seed deleted (G-CSF no seed) (mRNA sequence is shown in SEQ ID NO: 6601; polyA tail of approximately 140 nucleotides not shown in sequence; 5'Cap, Capl) fully modified with 5-methylcytidine and pseudouridine (5mC/pU) or fully modified with pseudouridine (pU) was tested at a

concentration of 250 ng per well in 24 well plates. The 24 hours after transfection, the expression of G-CSF was measured by ELISA, and the results are shown in Table 33.

Table 33. G-CSF mirl22 expression

	Hela cells	Primary human Hepatocytes	Primary rat Hepatocytes
	Protein Expression (ng/mL)	Protein Expression (ng/mL)	Protein Expression (ng/mL)
G-CSF miR-122 1X (5mC/pU)	167.34	67.60	3.40
G-CSF miR-122 1X (pU)	292.18	116.18	25.63
G-CSF no seed (5mC/pU)	194.78	129.77	8.39
G-CSF no seed (pU)	335.78	462.88	84.93

Example 43. Expression of modified nucleic acids with mir-122 binding sites in hepatocytes

[001091] MicroRNA control gene expression through the translational suppression and/or degradation of target messenger RNA. Mir-122 binding site containing G-CSF mRNA was translationally regulated in hepatocytes.

[001092] Primary rat and human hepatocytes and Hela cells were seeded at a density of 200,000 per well in 500 ul cell culture medium (InVitro GRO medium from Celsis, Chicago, IL). G-CSF mRNA (G-CSF alpha) (mRNA sequence is shown in SEQ ID NO: 6599; polyA tail of approximately 140 nucleotides not shown in sequence; 5'Cap, Capl) fully modified with 5-methylcytidine and pseudouridine (5mC/pU), G-CSF mRNA having a miR-122 binding site in the 3'UTR (G-CSF miR-122-IX) (mRNA sequence is shown in SEQ ID NO: 6600; polyA tail of approximately 140 nucleotides not shown in sequence; 5'Cap, Cap 1) fully modified with 5-methylcytidine and pseudouridine (5mc/pU) or G-CSF mRNA with four miR-122 binding sites with the seed deleted (G-CSF no seed) (mRNA sequence is shown in SEQ ID NO: 6601; polyA tail of approximately 140 nucleotides not shown in sequence; 5'Cap, Capl) fully modified with 5-methylcytidine and pseudouridine (5mC/pU) was tested at a concentration of 250 ng per well in 24 well plates. 24 hours after transfection, the expression of G-CSF was measured by ELISA. The G-CSF drug (mRNA) levels and protein levels are shown in Table 34.

Table 34. G-CSF drug and protein levels

	Human Hepatocytes		Rat Hepatocytes	
	Drug (mRNA) level (unit normalized to HPRT)	**Protein expression (ng/ml)**	**Drug (mRNA) level (unit normalized to HPRT)**	**Protein expression (ng/ml)**
G-CSF alpha (5mC/pU)	43237.6	247.26	26615.88	784.6
G-CSF miR-122-1X (5mC/pU)	46340.9	74.07	20171.07	40.628
G-CSF no seed (5mC/pU)	70239.7	298.28	23170.47	894.06

Example 44. Microphysiological Systems

[001093] The polynucleotides, primary constructs and/or mmRNA of the present invention are formulated using one of the methods described herein such as in buffer, lipid nanoparticles and PLGA. These formulations are then administered to or contacted with microphysiological systems created from organ chips as described in International Publication Nos. WO2013086502, WO2013086486 and WO2013086505, the contents of each of which are herein incorporated by reference in its entirety.

Example 45. Translation Enhancing Elements (TEEs) in Untranslated Regions

[001094] The 5' and/or 3' untranslated regions (UTRs) in the signal-sensor polynucleotides, primary constructs and/or mmRNA described herein may include at least one translation enhancing element (TEE). Such TEE which may be included in the 5'UTR and/or 3'UTR include, but are not limited to, those listed in Table 35, including portion and/or fragments thereof. The TEE sequence may include at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or more than 99% of the TEE sequences disclosed in Table 35 and/or the TEE sequence may include a 5-30 nucleotide fragment, a 5-25 nucleotide fragment, a 5-20 nucleotide fragment, a 5-15 nucleotide fragment, a 5-10 nucleotide fragment of the TEE sequences disclosed in Table 35.

Table 35. TEE Sequences

TEE	Sequence	SEQ ID

Identifier		NO
TEE-001	MSCSGCNGMWA	6645
TEE-002	RNSGAGMGRMR	6646
TEE-003	RNSGAGMGRMRRR	6647
TEE-004	RMSCSGCNGMWR	6648
TEE-005	GCGAGAGAA	-
TEE-006	GGGAGCGAA	-
TEE-007	GCGAGAGGA	-
TEE-008	GCGAGCGGA	-
TEE-009	CGGAGCGAA	-
TEE-010	CGGAGCGGA	-
TEE-011	ACGAGAGGA	-
TEE-012	ACGAGCGGA	-
TEE-013	GACGAGAGGA	6649
TEE-014	GACGAGAGAA	6650
TEE-015	AGCGAGCG	-
TEE-016	AGGAGAGGA	-
TEE-017	GCCGAGAGA	-
TEE-018	CGAGAGGCA	-
TEE-019	GAGAGGAGC	-
TEE-020	CGCGGCGGA	-
TEE-021	CGCCGCCGC	-
TEE-022	GCGGCTGAA	-
TEE-023	CCGGCTGAA	-
TEE-024	CGCCGCTGAA	6651
TEE-025	CGCCGCGGAA	6652
TEE-026	CGCCGCCGAA	6653
TEE-027	CCCGCGGAA	-
TEE-028	CCCGCCGAA	-
TEE-029	CCCGCTGAA	-
TEE-030	CCCGGCGGA	-
TEE-031	CGCGGCTGA	-
TEE-032	CGGCTGCTA	-
TEE-033	CCCGGCGGA	-
TEE-034	AGCCGCCGCA	6654
TEE-035	ACGCCGCCGA	6655
TEE-036	GGCATTCATCGT	6656
TEE-037	GCATTAGTATCT	6657
TEE-038	TCGGTTATTGTT	6658
TEE-039	TCCAATTGGGAA	6659
TEE-040	ATCTATTGGCCA	6660

TEE-041	TTACTGGGTGTT	6661
TEE-042	AGGGTGAAGGTC	6662
TEE-043	GGTGGGTGTGTC	6663
TEE-044	CGCTTCAATGCT	6664
TEE-045	TGCTTCAATGCC	6665
TEE-046	TGTGTCTTTGCA	6666
TEE-047	CACGGGGACAGC	6667
TEE-048	AAGCTGTACATG	6668
TEE-049	GATGGGGGCACA	6669
TEE-050	ATATGTGCCCTT	6670
TEE-051	TCCTTCTGGGTC	6671
TEE-052	GGTGGGTGTGTC	6672
TEE-053	GAATGGATGGGG	6673
TEE-054	CAXGTGATATTC	6674
TEE-055	AGGAGGGTTTGT	6675
TEE-056	TGGGCGAGTGGG	6676
TEE-057	CGGCTCACCAGT	6677
TEE-058	GGTTTCXATAAC	6678
TEE-059	GGTGGGTGTGTC	6679
TEE-060	TTACTGGGTGTT	6680
TEE-061	AAGTCTTTGGGT	6681
TEE-062	CCGGCGGGU	-
TEE-063	CCGGCGGG	-
TEE-064	CCGGCGG	-
TEE-065	CCGGCG	-
TEE-066	CCGGC	-
TEE-067	CGGCGGGU	-
TEE-068	GGGAGACGGCGGCGGTGGCGGCGCGGGCAGAGCAAG GACGCGGCGGATCCCACTCGCACAGCAGCGCACTCGG TGCCCCGCGCAGGGTCG	6682
TEE-069	AAAGAAATGGAATCGAAGAGAATGGAAACAAATGGA ATGGAATTGAATGGAATGGAATTGA ATGGAATGGGAACG	6683
TEE-070	AAAGAAATGGAATCGAAGAGAATGGAAACAAATGGA ATGGAATTGAATGGAATGGAATTGA ATGGAATGGGAACG	6684
TEE-071	AGACAGTCAGACAATCACAAAGAAACAAGAATGAAA ATGAATGAACAAAACCTTCAAGAAATATGGGATTATG AAGAGGCCAAATGT	6685
TEE-072	AAAAGGAAATACAAGACAACAAACACAGAAACACAA CCATCGGGCATCATGAAACCTCGTGAAGATAATCATCA GGGT	6686
TEE-073	AGACCCTAATATCACAGTTAAACGAACTAGAGAAGGA AGAGCAAACAAATTCAAAAGCTAGCGGAAAGCAAGA	6687

	AATAACTAAGACCAG	
TEE-074	AAAGACTTAAACATAAGACCTAAAACCATAAAAACCA CAGAAGAAAACATAGGCAATGCCATTCAGGACATAGG CATGGGCAAAGACTTC	6688
TEE-075	AGCAATAACCAAACAACCTCATTAAAAAGTAGGCAAA GGACATAAACAGACACTTTTCAAAAGAAGACATACAC GTGGCCAACAAACATATG	6689
TEE-076	AGAAAGAATCAAGAGGAAATGCAAGAAATCCAAAAC ACTGTAACAGATATGATGAATAATGAGGTATGCACTC ATCAGCAGACTCGACAT	6690
TEE-077	GCACTAGTCAGATCAAGACAGAAAGTCAACGAACAAA GAACAGACTTAAACTACACTCTAGA ACAAATGGACCTA	6691
TEE-078	AGCAGCCAACAAGCATATGAAATAATGCTCCACAACA CTCATCATCAGAGAAATGCAAATCA AAACCAAAAT	6692
TEE-079	AATATACGCAAATCAATAAATGTAATCCAGCATATAA ACAGTACTAAAGACAAAAACCACAT GATTATCTCAATAGATGCAGAAAAGGCC	6693
TEE-080	ATGTACACAAATCAATAAATGCAGTCCAGCATATAAA CAGAACCAAACACAAAAACCACATG ATTATCTCAATAGATGCAGAAAAGGCCTTT	6694
TEE-081	TATACCACACAAATGCAAAAGATTATTAGCAACAATT ATCAACAGCAATATGTCAACAAGTT GACAAACCTAGAGGACATGGAT	6695
TEE-082	AAACACACAAAGCAACAAAAGAACGAAGCAACAAAA GCATAGATTTATTGAAATGAAAGTA CATTCTACAGAGTGGGGGCAGGCT	6696
TEE-083	GAAATCATCATCAAACGGAATCGAATGGAATCATTGA ATGGAATGGAATGGAATCATCATGG AATGGAAACG	6697
TEE-084	AACAGAATGGAATCAAATCGAATGAAATGGAATGGAA TAGAAAGGAATGGAATGAAATGGA ATGGAAAGGATTCGAATGGAATGCAATCG	6698
TEE-085	TACAAAGAACTCAAACAAATCAGCAAGAACAAAAACA ATCCCAACAAAATGTTGGACAAAG ACATGAATAGACAATTCTCGAAAGAAGATGTACAAAT GGCT	6699
TEE-086	TGTTGAGAGAAATTAAACAAAGCACAGATAAATGGAA AAACGTGTTCATAGATTGAAAGACT TCATGTTGTATGGTGTC	6700
TEE-087	AAACGATTGGACAGGAATGGAATCACCATCGAATGGA AACGAATGGAATCTTCGAATGGAAT TGAATGAAATTATTGAACGGAATCAAATAGAATCATC ATTGAACAGAATCAAATTGGATCAT	6701
TEE-088	AACAATAAACAAACTCCAACTAGACACAATAGTCAAA TTGCTGAAAATGAAATATAAAGGAA CAATCTCGATGGTAGCCCAAGGA	6702
TEE-089	AAATCAATAAATGTAATTCAGCATATAAACAGAACCA AAGACAAAAACCACATGATTATCTC	6703

	AATAGATGCAGAAAAGGCCTTT	
TEE-090	GCTCAAGGAAATAAAATAGGACACAAAGAAATGGAA AAACATTCCATACTCATGGATAGAA AGAATCAATATCATGAAATGGCC	6704
TEE-091	AACATACGCAAATCAATAAATGTAATCCAGCATATAA ACAGAACCAAAGACAAAAACCACAT GATTATCTCAATAGATGCAGAAAAGGCC	6705
TEE-092	AACAATCACTAGTCCTTAAGTAAGAGACAACACCTTTT GTCACACACAGTTTGTCCTAACTTT ATCTTGGTAATTGGGGAGACC	6706
TEE-093	AGAAAACACACAGACAACAAAAAACACAGAACGACA ATGACAAAATGGCCAAGC	6707
TEE-094	ACACAACAACCAAGAAACAACCCCATTAAGAAGTGGG AAAAATACATGAATAAACACATCTC AAAAGAAGACAAACAAGTGGCTAAC	6708
TEE-095	ACAGCAGAAAACGAACATCAGAAAATCACTCTACATG ATGCTTAAATACAGAGGGCAAGCAA CCCAAGAGAAAACACCACTTCCTAAT	6709
TEE-096	GAATAGAACAGAATGGAATCAAATCGAATGAAATGGA ATGGAATAGAAAGGAATGGAATGA AATGGAATGGAAAGGATTCGAATGGAATG	6710
TEE-097	TAAGCAGAGAAAATATCAACACGAAAATAATGCAAGG AGAAAAATACAGAACAATCCAAAA TGTGGCC	671 1
TEE-098	GAACAATCAATGGAAGCAGAAACAAATAAACCAAGGT GTGCATCAAGGAATACATTCACGC ATGATGGCTGTATGAGTAAAATG	6712
TEE-099	GATCAATAAATGTAATTCATCATATAAACAGAGAACT AAAGACAAAAACACATGATTATCGC AATACATGCAGAAAAGGCC	6713
TEE- 100	GACAAGAGTTCAGAAAGGAAGACTACACAGAAATACG CATTTTAAAGTCACTGACATGGAGA TGACACTTAAAACCATGAACATGGATGGG	6714
TEE- 101	AAGCAAAGAAAGAATGAAGCAGCAAAAGAACGAAAG CAGGAATTTATTGAAAACCAAAGTA CACTCCACAGTATGGGAGCGGACCCGAGCA	6715
TEE- 102	ACCAACATAAGACAAAGAAACATCCAGCAGCTGCCTA TGGCAAAAGATTACAATGTGTCAAA CAAGAGGGCAATG	6716
TEE- 103	GGACAAATTGCTAGAAATAAACAAATTACCAAAAATG ATTCAAGTAGAGACAGAGAATCAA AATAGAACTACACATAAGTGGGCCAAG	6717
TEE- 104	AACATAATCCATCAAATAAACAGAACCAAAGACAAAA ACCACATGATTATCTCAATAGATGC AGAAAAGGCCTTC	6718
TEE- 105	AAAATCAATATGAAAACAAACACAAGCAGACAAAGA AAATTGGGCAAAAGGTTTGAGCAGA CACTTCACCAAAGAAGTACAAATGGCAAATCAGCA	6719
TEE- 106	AACCAAATTAGACAAATTGGAAATCATTACACATAAC AAAAGTAATAAACTGTCAGCCTCAG	6720

	TAGTATTCATTGTACATAAACTGGCC	
TEE-107	AAGGAATTTAAGCAAATCAACAAGCAAAACCAAAATA ATCCCATTAAAAAGTGGGTAAAGG ACATGAATACACACTTGTCAATAGAGGACATTCAAGT GGCCAAC	6721
TEE-108	TAACCTGATTTGCCATAATCCACGATACGCTTACAACA GTGATATACAAGTTACATGAGAAAC ACAAACATTTTGCAAGGAAACTGTGGCCAGATG	6722
TEE-109	AACTAACACAAGAACAGAAAACCAAACATCACATGTT CTCACTCATAAGCGGGAGCTGAACA ATGAGAACACACGGACACAGGGAGAGGAACATG	6723
TEE-110	TAAACTGACACAAACACAGACACACAGATACACACAT ACATACAGAAATACACATTCACACA CAGACCTGGTCTTTGGAGCCAGAGATG	6724
TEE-111	ATCAACAGACAACAGAAACAAATCCACAAAGCACTTA GTTATTAGAACTGTCATACAGACTG TACAACAACCACATTTACCAT	6725
TEE-112	AAATAAGCCAACGGTCATAAATTGCAAAGCCTTTTACA ATCCAAACATGATGGAAACGATAT GCCATTTTGAAGGTGATTTGAAAAGCACATGGTTT	6726
TEE-113	AAACAGTTCAAAAATTATTGCAACAAAATGAGAGAGA TGAGTTTATCTTGCAAACTAATGGA TGGTAGCAGTGACAGTGGCAAAACGTGGTTTGATTCT	6727
TEE-114	TAAGCAACTTCAGCAAAGTCTCAGGATACAAAATCAA TGTACAAAAATCACAAGCATTCTTA TACACCAACAACAGACAAACAAGAGTGCCAAATCATG	6728
TEE-115	AGCAAACAAACAAACAAACAAACAAACTATGACAGG AACAAAACGTCACATATCAACATTA ACAAAGAATGTAAACAGCCTAAATGCTTCACTTAAAA GTTATAGACAGGGGCTGGGCATGGT GGCTCACGCC	6729
TEE-116	GGAAATAACAGAGAACACAAACAAATGGGAAAACATT CCATGTTCATGGATAGGAAGAATC AATATTGTGAAAATGGCCATACT	6730
TEE-117	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAATG TACAAAAATCACAAGCATTCTTATA CACCAATAACAGACAAACAGAGAGCC	6731
TEE-118	AGATAAGAATAAGGCAAACATAGTAATAGGGAGTTCA TGAATAACACACGGAAAGAGAACT TACAGGGCTGTGATCAGGAAACG	6732
TEE-119	AGGAAATAAAAGAAGACACAAACAAATGGAAGAACA TTCCATGCTTATGGATAGGGAGAAT CAGTATCGTGAAAATGGCCATACT	6733
TEE-120	AACATACGAAAATCAATAAACGTAATCCAGCATATAA ACAGAACCAAAGACAAAAACCACA TGATTATCTCAATAGATGCAGAAAAGGCCTTT	6734
TEE-121	AATGGACTCGAATGAAATCATCATCAAACGGAATCGA ATGGAATCATTGAATGGAATGGAAT GGAATCATCATGGAATGGAAACG	6735
TEE-122	AAGATTTAAACATAAGACCTAAAACGACAAAAATCCT	6736

	AGGAGAAAACCTAAGCAATACCATT CAGGACATAGGCATGGGCAAAGACTTCATG	
TEE- 123	TAATGAGAAGACACAGACAACACAAAGAATCACAGAA ACATGACACAGGTGACAAGAACAG GCAAGGACCTGCAGTGCACAGGAGCC	6737
TEE- 124	TAAACGTTAGACCTAAAACCATAAAAACCCTAGAAGA AAACCTAGGCATTACCATTCAGGAC ATAGGCATGGGCAAGGAC	6738
TEE- 125	GAATTGAATTGAATGGAATGGAATGCAATGGAATCTA ATGAAACGGAAAGGAAAGGAATGG AATGGAATGGAATG	6739
TEE- 126	GTAATGGAATGGAATGGAAAGGAATCGAAACGAAAG GAATGGAGACAGATGGAATGGAATG GAACAGAG	6740
TEE- 127	AGAGAAATGCAAATCAAAACCACAATGGAATACCATC TCACGCCAGTCAGAATGGCAATTAT TAAAAAATCACAACAATTAATGATGGCAAGGCTGTGG	6741
TEE- 128	AACATACACAAATCAATAAACGTAATCCAGCTTATAA ACAGAACCAAAGACAAAAACCACAT GATTATCTCAATAGATGCGGAAAAGGCC	6742
TEE- 129	TAAACAGAACCAAAGACAAAAATCACATGATTATCTC AATAGATGCAGAAAAGGCC	6743
TEE- 130	AATGGAATGCAATCGAATGGAATGGAATCGAACGGAA TGGAATAAAATGGAAGAAAACTGG CAAGAAATGGAATCG	6744
TEE- 131	AGATAAAAAGAACAGCAGCCAAAATGACAAAAGCAA AAAGCAAAATCGTGTTAGAGCCAGG TGTGGTGATGTGTGCT	6745
TEE- 132	AGGAAAGTTTTCAATATGAGAAAGATACAAACCAACA GAATAAGCAAACTGGATAAACAGA AAATACAGAGAGAGCCAAGG	6746
TEE- 133	GCAATCTCAGGATACAAAATCAATGTGCAAAAATCAC AAGCATTCTCATACACCAATAACAG ACAAACAGAGCCAAATCATG	6747
TEE- 134	AGCATTCATATCTTGCAGTGTTGGGAAAGAGTGAGAG GTTGTGATGTCAAGAAGGATAGGTC AGAAGTGGAAGGTATGGGGGATTGTGCCTGCTGTCAT GGCT	6748
TEE- 135	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAATG TGCAAAAATCACAAGCATTCTTATA CACCAATAACAGACAAACAGAGAGCC	6749
TEE- 136	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAATG TGCAAAAATCACAAGCATTCTTATA CACCAACAACAGACAAACAGAGAGCC	6750
TEE- 137	TAAGCCGATAAGCAACTTCAGCAAAGTCTCAGGAGAC AAAATCAATGTGCAAAAAATCACAA GCATTCTTATACACTAATAACAGACAAACAGAGAGCC AAATCATG	6751
TEE- 138	AACGTGACATACATACAAAAAGTTTTTAGAGCAAGTG AAATTTTAGCTGCTATATGTTAATTG	6752

	GTGGTAATCCC	
TEE- 139	TACGCAAATCGATAAATGTAATCCAGCATATAAACAG AACCAAAGACAAAAACCACATGATT ATCTCAATAGATGCAGAAAAGGCC	6753
TEE- 140	GCAATCGAATGGAATGGAATCGAACGGAATGGAATAA AATGGAAGAAAACTGGCAAGAAAT GGAATCG	6754
TEE- 141	TTGAATCGAATGGAATCGAATGGATTGGAAAGGAATA GAATGGAATGGAATGGAATTGACTC AAATGGAATG	6755
TEE- 142	TAAAGAAAAACAAACAAACAGAAATCAATGAAAATCC CATTCAAAGGTCAGCAACCTCAAA GACTGAAGGTAGATAAGCCCACAAGGATG	6756
TEE- 143	GTCATATTTGGGATTTATCATCTGTTTCTATTGTTGTTG TTTTAGTACACACAAAGCCACAATA AATATTCTAGGCT	6757
TEE- 144	AAAAGTACAGAAGACAACAAAAAATGAGAGAGAGAA AGATAACAGACTATAGCAGCATTGG TGATCAGAGCCACCAG	6758
TEE- 145	AACCCACAAAGACAACAGAAGAAAAGACAACAGTAG ACAAGGATGTCAACCACATTTTGGA AGAGACAAGTAATCAAACACATGGCA	6759
TEE- 146	AAAGACCGAAACAACAACAGAAACAGAAACAAACAA CAATAAGAAAAAATGTTAAGCAAAA CAAATGATTGCACAACTTACATGATTACTGAGTGTTCT AATGGT	6760
TEE- 147	AATCAGTAAACGTAATACAGCATATAAACAGAACCAA AGACAAAAACCACATGATTATCTCA ATAGATGCAGAAAAGGCC	6761
TEE- 148	AAGCAACTTCAGCAAAGTCTCAGGACACAAAATCAAT ATGCGAAAATCACAAGCATTCCTAT ACACCAATAATAGACAAACAGAGAGCCAAATCATG	6762
TEE- 149	AGCAACTTCAGCAAAATCTCAGGATACAAAATCAATG TACAAAAATCACAAGCATTCTTATA CACCAACAACAGACAAACAGAGAGCC	6763
TEE- 150	TAATGCAAACTAAAACGACAATGAGATATCAATACAT AACTACCAGAAAGGCTAACAAAAAA ACAGTCATAACACACCAAAGGCTGATGAGTGAGGATG TGCAG	6764
TEE-151	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCGATG TGCAAAAATCACAAGCATTCTTATA CACCAATAACAGGCAAACAGAGAGCC	6765
TEE- 152	GATATATAAACAAGAAAACAACTAATCACAACTCAAT ATCAAAGTGCAATGATGGTGCAAAA TGCAAGTATGGTGGGGACAGAGAAAGGATGC	6766
TEE- 153	AAGACAGAACACTGAAACTCAACAGAGAAGTAACAAG AACACCTAAGACAAGGAAGGAGAG GGAAGGCAGGCAG	6767
TEE- 154	TAAGACACATAGAAAACATAAAGCAAAATGGCAGATG TAAATGCAACCTATCAATCAAAACA	6768

	TTACGAATGGCTT	
TEE- 155	TGAAACAAATGATAATGAAAATACAACATACCAAACA TACGAGATACAGTAAAAGCAGTACT AAGATGCAAGTATATATTGCTACAAGTGCCTAC	6769
TEE- 156	AATGTAATCCAGCATATAAACAGAGCCAAAGACAAAA ACCACATGATTATCTCAATAGATGC AGAAAAAGCCTTTGACAAAATTCAACAACCCTTCATGC TAAAAACTCTCAATAAATTAGGTAT TGATGGGACG	6770
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TEE- 158	AAGGATTCGAATGGAATGCAATCGAATGGAATGGAAT CGAACGGAATGGAATAAAATGGAA GAAAACTGGCAAGAAATGGAATCG	6772
TEE- 159	GATCATCAGAGAAACAGAGAAATGCAAATTAAAACCA CAATGAGATACTATCTCCACACAAG TCAGAATGGCTAT	6773
TEE- 160	ATCAAAAGAAAAGCAACCTAACAAATACGGGAAGAAT ATTTGAATAGACATTTCACAGGAAA AGATATATGAATGGCCAAAAAGCAAATGAAAAG	6774
TEE- 161	AACAGCAATGACAATGATCAGTAACAACAAGACTTTT AACTTTGAAAAAATCAGGACC	6775
TEE- 162	AAGAGCCTGAATAGCTAAAGTGATCATAAGCAAAAAG AACAAAGTCGGAAGCATCACATTAC CTGACTTCAAACTATACTCAAAGGCTATG	6776
TEE- 163	ACTCAGGAAAAATAACGAATCCAACTCACAGGAGAAA GAAGTACAAACCAGAAACCAATTT CAAATTACAAGGACCAGAATACTCATGTTGGCTGGCC AGT	6777
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TEE- 166	AGCAACTTCAGCAAAGACTCAGGATACAAAATCAATG TGCAAAAATCACAAGCATTCTTATA CACCAATAACAGACAGAGAGCCAAAT	6780
TEE- 167	TGGGATATGGGTGAAAGAACAAGTTTGCAGAAAAGAT ACAGTGAATTATGGACCATGAGTTC GGGAAAGAAGGGTAGGACTGCG	6781
TEE- 168	AGCAGTGCAAGAACAACATAACATACAAGTAAACAAA CACATGGGGCCAGGTAATAAAAAG TCAGGCTCAAGAGGTCAG	6782
TEE- 169	AAGGAAAAGTAAAAGGAACTTAACACCTTCAAGAAAA GACAGACAAATAACAAAACAGCAG TTTGATAGAATGAGATATCAGGGGATGGCA	6783
TEE- 170	GCTAGTTCAACATATGCAAATCAATAAACGTAATCCAT CACATAAACAGAACCAATGACAAA	6784

	AACCACGATTATCTCAATAGATGCAGAAAAGGCC	
TEE-171	AACATCACTGATCATTAGAAACACACAAATCAAAACC ACAATAAGATACCATCTAACACCAG TCACAATGGCTATT	6785
TEE-172	AGAGCATCCACAAGGCCCAATTCAAAGAATCTGAAAT AATGTATTGTTACTGCAACAGTTGTG AGTACCAGTGGCATCAG	6786
TEE-173	GGAATAACAACAACAACAACCAAAAGACATATAGAAA ACAAACAGCACGATGGCAGATGTA AAGCCTACC	6787
TEE-174	AAACGCAGAAACAAATCAACGAAAGAACGAAGCAAT GAAAGACAAAGCAACAAAAGAATG GAGTAAGAAAGCACACTCCACAAAGTGGAAGCAGGCT GGGACA	6788
TEE-175	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAATG TGCAAAAATCACAAGCATTCCTATA CACCAACAACAGACAAACAGAGAGCC	6789
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TEE-181	AGAAAGGATTCGAATGGAATGAAAAAGAATTGAATGG AATAGAACAGAATGGAATCAAATC GAATGAAATGGAATGGAATAGAAAGGAATGGAATG	6795
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TEE-183	AAGAGTATTGAAGTTGACATATCTAGACTGATCAAGA ACAAAGACAAAAGGTACAGATTATC AAGAAAATGAGCGGGCAAAGCAAGATGGCC	6797
TEE-184	AGTAGAATTGCAATTGCAAATTTCACACATATACTCAC ACACAAGTACACACATCCACTTTTA CAACTAAAAAAACTAGCACCCAGGACAGGTGCAGTGG CT	6798
TEE-185	TGAATGCTATAGAGCAGTAAAAACAAATAAATGAACT ACATTACAGCTACTTACAACCATAT GAAAGAATATAACCATAACAATGATGAGTGGACAAAA GCTAAGTGTGAAAGAATGCATAGT	6799

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TEE-187	TAAAAGTGTGCTCAACATCATTGATCATCAGAGAAATG CAAATCAAAACTACAATGAGATAT CATCTCATCCCAGTCAAAGTGGCT	6801
TEE-188	TCAGACCATAGCAGATAACATGCACATTAGCAATACG ATTGCCATGACAGAGTGGTTGGTG	6802
TEE-189	ACAAACAATCCAATTCGAAAATGGGCAAGATATTTCA CCAAAGACATGAGCTGATATTTCAC	6803
TEE-190	AGGAAAAACAACAACAACAACAGGAAAACAACCTCA GTATGAAGACAAGTACATTGATTTAT TCAACATTTACTGATCACTTTTCAGGTGGTAGGCAG	6804
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TEE-192	ATACAACTAAAGCAAATATAAGCAACTAAAGCAACAG TACAACTAAAGCAAAACAGAACAA GACTGCCAGGGCCTAGAAAAGCCAAGAAC	6806
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TEE-194	AGAGAGTATTCATCATGAGGAGTATTACTGGACAAAT AATTCACAAACGAACAAACCAAAGC GATCATCTTTGTACTGGCTGGCTA	6808
TEE-195	AGTAAATCACCATAAAGAAGGTAAGAGTTCATTCACA AAAACAACAAACTGAAGAATCAGGC CATAGTA	6809
TEE-196	AAAATAGAATGAAAGAGAATCAAATGGAATTGAATCG AATGGAATCGAATGGATTGGAAAG GAATAGAATGGAATGGAATGGAATG	6810
TEE-197	AAAAGATGCAAAAGTAGCAAATGCAATGTTAAAACAA GCAAAGAAAGAATCAGGTGGACCA CATAGTGCAGTGCTTCTC	681 1
TEE-198	TTCACAGCAGCATTACGCACAATAGCCAGAAGGTGGG AACAGACAAAATGCCTTTTGATGGG	6812
TEE-199	CCATAACACAATTAAAAACAACCTAAATGTCTAATAG AAGAACACTGTTCAGACCGGGCATG GTGGCTTATACC	6813
TEE-200	TGGATTTCAGATATTTAACACAAAATAGTCAAAGCAG ATAAATACTAGCAACTTATTTTTAAT GGGTAACATCATATGTTCGTGCCTT	6814
TEE-201	ATCATTGAATGCAATCACATGGAATCATCACAGAATG GAATCGTACGGAATCATCATCGAAT GGAATTGAATGGAATCATCAATTGGACTCGAATGGAA ACATCAAATGGAATCGATTGGAAGT GTCGAATGGACTCG	6815
TEE-202	AGAAACAGCCAGAAAACAATTATTACCTACAGCATTA	6816

	AAACTATTCAAATGACAGCATATTTT TCAGCAGAAATCATGAAGGCCAGAAGGACGTGTCAT	
TEE-203	AAAATGATCATGAGAAAATTCAGCAACAAAACCATGA AATTGCAAAGATATTACTTTTGGGA TGGAACAGAGCTGGAAGGCAAAGAG	6817
TEE-204	AACCACTGCTCAAGGAAATAAGAGAGAACACAAACAA ATGAAAAAACATTCCATGCTCATGG ATAGGAAGAATCAG	6818
TEE-205	TACTCTCAGAAGGGAAGCAGATATTCAGCATAAATCA TATTGTTTGTACAAAGAGTCTGGGCA TGGTGAATGACACT	6819
TEE-206	TATAGTTGAATGAACACACATACACACACACATGCCA CAAAACAAAAACAAAGTTATCCTCA CACACAGGATAGAAACCAAACCAAATCCCAACACATG GCAAGATGAT	6820
TEE-207	GCTCAAAGAAATCAGAAATGACACAAGCAAATGGAAA AACATGCCATGTTCATGAATATGAA GAATCAATATTGTTAAAATGGCCATACTGCTCA	6821
TEE-208	GGATACAAAATCAATGTACAAAAATCACAAGCATTCT TATACACCAATAACAGACAAACAGA GAGCC	6822
TEE-209	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAATG TACAAAAATCACAAGCATTCTTATA CACCAACAACAGACAAACAGAGAGCC	6823
TEE-210	AGGAGAATAGCAGTAGAATGACAAAATTAGATTTTCA CATGAAACTTGATGACAGTGTAGGA AATGGACTGAAAGGACAAGAC	6824
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TEE-213	AAGAAACAATCAAAAGGAAGTGCTAGAAATAAAACAC ACTGTAATAGAAAAGAAGAATGCC TTATGGGCTTATCAATAGACTAGACATGGCCAGG	6827
TEE-214	AAAGAAAGACAGAGAACAAACGTAATTCAAGATGACT GATTACATATCCAAGAACATTAGAT GGTCAAAGACTTTAAGAAGGAATACATTCAAAGGCAA AAAGTCACTTACTGATTTTGGTGGA GTTTGCCACATGGAC	6828
TEE-215	AGCAACTTCAGCAAAGTTTCAGGATACAAAATCAATG TGCAAAAATCACAAGCATTCTTATA CACCAACAACAGACAAACAGAGAGCC	6829
TEE-216	AGAATCAAATGGAATTGAATCGAATGGAATCGAATGG ATTGGAAAGGAATAGAATGGAATG GAATGGAATG	6830
TEE-217	AAACAGAACCACAGATATCTGTAAAGGATTACACTAT	6831

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TEE-218	AAAAAATGTTCAACATCACTAGTCAGCAGAGAAATGC AAATCAAAATCACAATGAGATAACT TCTCACACCAGACAGCATGGC	6832
TEE-219	GAATCCATGTTCATAGCACAACAACCAAACAGAAGAA ATCACTGTGAAATAAGAAACAAAGC AAAACACAGATGTCGACACATGGCA	6833
TEE-220	AGGATACAAAATCAAAGTGCAAAAATCACAAGCATTC TTATACACCAATAACAGACAAACAG AGAGCC	6834
TEE-221	AACAGATTTAAACAAACCAACAAGCAAAAAACGAACA ACTCCATTCAAACATGGACAAAAG ACACGAACAGACACTTTTCAAAGAAGACATACATGTG GCC	6835
TEE-222	AAAGACAATATACAAATGGCCAATAAGCACATGAAAA GACGCTCAACATCCTTAGTCGTTAA GGCAATGCAAATCAAAACCACAATG	6836
TEE-223	TAAACAACGAGAACACATGAACACAAAGAGGGGAAC AACAGACACCAAGACCTTCTTGAGG GTGGAGGATGGGAGGAGGGAG	6837
TEE-224	GGTTCAACTTACAATATTTTGACTTGACAACAGTGCAA AAGCAATACACGATTAGTAGAAAC ACACTTCCAATGCCCATAGGACCATTCTGC	6838
TEE-225	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAATG AGCAAAAATCACAAGCATTCTTACA CACCAATAACAGACAAACAGAGAGCC	6839
TEE-226	AATCCAGCATATAAACAGAACCAAAGACAAAAACCAC ATGATTATCTCAATAGATGCAGAAA AGGCC	6840
TEE-227	TGAAAATACAAATGACCATGCAAGTAATTCCGCAGGG AGAGAGCGGATATGAACAAACAGA AGAAATCAGATGGGATAGTGCTGGCGGGAAGTCA	6841
TEE-228	GCAAATGATTATAAGTGCTGTTATAGAAACATTCAAAG ACCAGAAAAGGACCACAATGGCTG ACCAC	6842
TEE-229	AGTCAATAACAAGAAGACAAACAACCCAATTACAAAA TGGGATATGAATTTAATAGATGTTA CTCCAAGGAAGATACACAAATGGCCAAC	6843
TEE-230	ATGGTTAAAACTCAACAATGAAAACACAAACAGCGCA ATTTAAAAATGGGCAAAATGACAG GCCAGACCCAGTGGCTCATGCG	6844
TEE-231	TAACTACTCACAGAACTCAACAAAACACTATACATGC ATTTACCAGTTTATTATAAAGATACA AGTCAGGAACAGCCAAATGGAAGAAATGTAAATGGCA AG	6845
TEE-232	AACAGACCATAAATAAACACAGAAGACACACGAGTGT AAAGTCAGTGCCCCGCTGCGAATTA AATCGGGGTGATGTGATGGCGAGTGAGTGGGTAGTT	6846

TEE-233	GAATAGAATAGAATGGAATCATCGAATGGAATCGAAT GGAATCATCATGATATGGAATTGAG TGGAATC	6847
TEE-234	GGAATCTATAATACAGCTGTTTATAGCCAAGCACTAAA TCATATGATACAGAAAACAAATGC AGATGGTTTGAAGGGTGGG	6848
TEE-235	AAGATAGAGTTGAAACAGTGGACAATTAAAGAGTAAT TTGGAAGAATGGTGAAATTACAGCC ATGCTTTGAATCAGGCGGGTTCACTGGC	6849
TEE-236	TGAAAAGAAGAATGACCATAAGCAAGCAGATGAAAA ACAAAACAGAATTTTTACAGACGTCT TGGACTGATATCTTGGGC	6850
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TEE-238	AGGAAAAGAAAGAAATAGAAAATGCGAAATGGTAAG AAAAAACAGCATAATAAACATTTGT ATGGTGTTGATGGACAATGCATT	6852
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TEE-240	GCAAAACACAAACAACGCCATAAAAAACTGGGCAAAG GATATGAACAGACATTTTTCAAAAC AAAACATACTTATGGCCAAC	6854
TEE-241	AACAAAATTGAACAACATGCAAAGAAACATAAACGAA GCAATGAAAGTGTGCAGATCCACT GAAATGAAAGTGCTGTCCAGAGTGGGAGCCAGCTCGA GA	6855
TEE-242	GAATGGAATCAACATCAAACGGAATCAAACGGAATTA TCGAATGGAATCGAAGAGAATCATC GAATGGCCACGAATGGAATCATCTAATGGAATGGAAT GGAATAATCCATGG	6856
TEE-243	TACAAGAAAATCACAGTAACATTTATAAAACACAGAA GTGTGAACACACAGCTATTGACCTT GAAAACAGTGAAAGAGGGTCAGCTGTAGAACTAAGAC ATAAGCAAAGTTTTTCAATCAAGAA TACATGGGTGGCC	6857
TEE-244	AAGAATTGGACAAAACACACAAACAAAGCAAGGAAG GAATGAAAGGATTTGTTGAAAATGA AAGTACACTCCACAGTGTGGGAGCAG	6858
TEE-245	ACAGTTAACAAAAACCGAACAATCTAATTACGAAATG AACAAAAGATATGAACAGACATTTC ACCCGAGAGTATACAGGGGCCAGGCATGGT	6859
TEE-246	AAACGCACAAACAAAGCAAGGAAAGAATGAAGCAAC AAAAGCAGAGATTTATTGAAAATGA AAAATACACTCCACAGGGTGGG	6860
TEE-247	CACCATGAGTCATTAGGTAAATGCAAATCAAAACCAC AATGAAATACTTCACACCCATGAAG ATGGCTATAATAAAAAAACAGACA	6861

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TEE-254	GAACGATTTATCACTGAAAATTAATACTCATGCAAGTA GTAAACGAATGTAATGACCATGAT AAGGAGACGGACGGTGGTGATAGT	6868
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TEE-261	TATGCAAATCAATAAACATAATCCATCACATAAACAG AAACAAAGACAAAATGACATGATTA TCTCAATAGATGCAGAAAAGGCC	6875
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TEE-264	AGGAAAATGCAAATCAGAACGACTATAACACACCATC	6878

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TEE-266	AAAGGAAAACTACAAAACACTGCTGAAAGAAATCATT GACAACACAAACAAATGGAAACAC ATCCCAAGATCATGGGTGGGTGGAATCAAT	6880
TEE-267	ACACACATACCAACAGAACATGACAAAAGAACAAAAC CAGCCGCATGCATACTCGATGGAG ACAAAGGTAACACTGCAGAATGGTGAAGGAAGAACAG TCATTTTAATGACAGTGTTGGCT	6881
TEE-268	AACTAAGACAACAGATTGATTTACACTACTATTTTCAC ACAGCCAAAAATATCACTATGGCAA TCGTCAAAAGGTCAATTCAAAGATGGGACAGT	6882
TEE-269	GATCAGCTTAGAATACAATGGAACAGAACAGATTAGA ACAATGTGATTTTATTAGGGGCCAC AGCACTGTTGACTCAAGTACAAGTTCTGACTCATGTAG AACTAACACTTTT	6883
TEE-270	GAATGGAATCAAATCGAATGAAATGGAATGGAATAGA AAGGAATGGAATGAAATGGAATGG AAAGGATTCGAAT	6884
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TEE-278	AAAACACACAAACATACATGTGGATGCACATATAAAC ATGCACATACACACACACATAAATG CACAAACACACTTAACACAAGCACACATGCAAACAAA CACATGG	6892
TEE-279	TAGAAGGAATTTGATACATGCTCAGAAATACAGGCAA AGGAAGTAGGTGCCTGCCAGTGAAC ACAGGGGAACTATGGCTCCTA	6893

TEE-280	TGACTAAACAGAGTTGAACAAGAACAAAAAGCAAATT TGCAGAAATGAAATACATACTAATT GAAAGTCCATGGACAGGCTCAACAGATGATATAGATA CAGCTAAAGAGATAATTAGTGAAAT GGATCAG	6894
TEE-281	AAGTAATAAGACTGAATTAGTAATACAAAGTGTCTCA ACAAAGAAAATTGCGGGACTGTTCA TGCTCATGGACAGGAAGAATCAATATCATGAAAATGG CC	6895
TEE-282	ACAGACAGAGATTTAAAACAATAAACAAGCAGTAAGC AAACACAGATAACAAAATGACATG ATCCAACAAATACTCAGAAGGAGACTTAGAAATGAAT TGAGGGTC	6896
TEE-283	AGAAAAAAACAAACAGCCCATTAAAAGGTAGACAAA GGACATGAACACTTTTCAAAAGAAG ACATACATGTGGCCAAACAGCATG	6897
TEE-284	AAAAATGACCAGAGCAATAGAATGCATTGACCAGATA AAGACCTTCACGTATGTTGAACTAA AATGTGTGGTGCAGGTG	6898
TEE-285	AATCAGTCTAGATCTTAAAGGAACACCAGAGGGAGTA TTTAAATGTGCCCAATAAGCAAGAA TTATGGTGATGTGGAAGTA	6899
TEE-286	GAATGGAATGGAAAGGAATCGAAACGAAAGGAATGG AGACAGATGGAATGGAATGGAACAG AGAGCAATGG	6900
TEE-287	GGAATGGAATGAACACGAATGTAATGCAACCCAATAG AATGGAATCGAATGGCATGGAATAT AAAGAAATGGAATCGAAGAGAATGGAAACAAATGGA ATGGAATTG	6901
TEE-288	AGGACATGAATAGACAATTCTCAAAAGAAGATACACA AGTGGCAAACAAACACATGAAAAA AGACTCAACATTAGTAATGACCATGGAAATGCAAATC	6902
TEE-289	TCCAGTCGATCATCATATAGTCAGCACTTATCATACAC CAAGCCGTGTGCAAGGAAAGGGAA TACAACCATGAACATGATAGATGGATGGTT	6903
TEE-290	TACAGATAAGAAAATTGAGACTCAAGAGTATTACATA AATTGTTTCAGCTACCACAGCAAAA AATGGTATGGTTGGGAATCAAGCTCAGGG	6904
TEE-291	AGCCTATCAAAAAGTGGGCTAAGAATATGAATACACA ATTCTCAAAAGAAGATATACAAATG GGCAACAAACATATGAAAACATACTCAACATCACTAA TGATCAGGGAAATG	6905
TEE-292	GAAAATGAACAATATGAACAAACAAACAAAATTACTA CCCTTACGAAAGTACGTGCATTCTA GTATGGTGACAAAAAGGAAAG	6906
TEE-293	ACATACGCAAATCAATAAACATAATCCATCACATAAA CAGAACCAAAGACAAAAATCACATG ATTATCTCAATAGATGCAGAAAAGGCCTTCGAC	6907
TEE-294	AAGAGTATCAACAGTAAATTACATTAGCAGAAGAATC AACAAACATGAAAATAGAAATTATG	6908

	GTAGCCAAAGAACAG	
TEE-295	AATCGAATGGAATCAACATCAAACGGAAAAAAACGGA ATTATCGAATGGAATCGAAGAGAATCATCGAATGGAC C	6909
TEE-296	GAAAGGAATAGAATGGAATGGATCGTTATGGAAAGAC ATCGAATGGGATGGAATTGACTCGAATGGATTGGACT GGAATG GAACGGACTCGAATGGAATGGACTGGAATG	6910
TEE-297	TAAGCAATTTCAGCAGTCTCAGGATACAAAATCAATGT GCAAAAATCACAAGCATTCTTATACACCAACAACAGA CAAAC AGAGAGCCAAATCG	691 1
TEE-298	AACGGAATCAAACGGAATTATCGAATGGAATCGAAGA GAATCATCGAATGGCCACGAATGGAATCATCTAATGG AATGG AATGGAATAATCCATGGACCCGAATG	6912
TEE-299	ACATCAAACGGAATCAAACGGAATTATCGAATGGAAT CGAAAAGAATCATCGAACGGACTCGAATGGAATCATC TAATGG AATGGAATGGAAG	6913
TEE-300	ATCGAATGGAATCAACATCAAACGGAAAAAAACGGAA TTATCAAATGGAATCGAAGAGAATCATCGAATGGACC	6914
TEE-301	GAATAATCATTGAACGGAATCGAATGGAAACATCATC GAATGGAAACGAATGGAATCATCATCGAATGGAAATG AAAGG AGTCATC	6915
TEE-302	CATCAAACGGAATCAAACGGAATTATCGAATGGAATC GAAAAGAATCATCGAACGGACTCGAATGGAATCATCT AATGGA ATGGAATGGAAGAATCCATGGACTCGAATG	6916
TEE-303	AAACGGAATCAAACGGAATTATCGAATGGAATCGAAG AGAATCATCGAATGGACTCGAATGGAATCATCTAATG GAATGG AATGGAAGAATCCATGG	6917
TEE-304	ATACACAAATCAATAAATGTAATCCAGCATATAAACA GAACCAAAGACAAAAACCATATGATTATCTCAATGGA TGCAGA AAAGGCC	6918
TEE-305	AATCGAATAGAATCATCGAATGGACTCGAATGGAATC ATCGAATGTAATGATGGAACAGTC	6919
TEE-306	TGGAATGGAATCATCGCATAGAATCGAATGGAATTAC CATCGAATGGGATCGAATGGTATCAACATCAAACGCA AAAAAA CGGAATTATCGAATGGAATCGAAGAGAATCTTCGAAC GGACCCG	6920
TEE-307	ATGGAATGGAATGGAATGGAATTAAATGGAATGGAAA GGAATGGAATCGAATGGAAAGGAATC	6921
TEE-308	GTCGAAATGAATAGAATGCAATCATCATCAAATGGAA TCCAATGGAATCATCATCAAATAGAATCGAATGGAAT CATCAA	6922

	ATGGAATCGAATGGAGTCATTG	
TEE-309	TGGAATTATCGAAAGCAAACGAATAGAATCATCGAAT GGACTCGAATGGAATCATCGAATGGAATGGAATGGAA CAG	6923
TEE-310	AAAGGAATGGAATGCAATGGAATGCAATGGAATGCAC AGGAATGGAATGGAATGGAATGGAAAGGAATG	6924
TEE-31 1	AATCTAATGGAATCAACATCAAACGGAAAAAAACGGA ATTATCGAATGGAATCGAAGAGAATCATCGAATGGAC C	6925
TEE-312	TACACAACAAAAGAAATACTCAACACAGTAAACAGAC AACCTTCAGAACAGGAGAAAATATTTGCAAATACATC TAACAA AGGGCTAATATCCAGAATCT	6926
TEE-313	TGCAATCCTAGTCTCAGATAAAACAGACATTAAACCA ACAAAGATCAAAAGAGACAAAGAAGGCCATTAC	6927
TEE-314	GAATCGAATGGAATCAACATCAAACGGAAAAAAACGG AATTATCGAATGGAATCGAAAAGAATCATCGAATGGA CC	6928
TEE-315	AATGGAATCGAATGGAATGCAATCCAATGGAATGGAA TGCAATGCAATGGAATGGAATCGAACGGAATGCAGTG GAAGG GAATGG	6929
TEE-316	GAACACAGAAAAATTTCAAAGGAATAATCAACAGGGA TTGATAACTAACTGGATTTAGAGAGCCAAGGCAAAGA GAATC AAAGCACAGGGCCTGAGTCGGAG	6930
TEE-317	AGTTGAATAGAACCAATCCGAATGAAATGGAATGGAA TGGAACGGAATGGAATTGAATGGAATGGAATGGAATG CAATG GA	6931
TEE-318	AACTCGATTGCAATGGAATGTAATGTAATGGAATGGA ATGGAATTAACGCGAATAGAATGGAATGGAATGTAAT GGAACG GAATGGAATG	6932
TEE-319	AAGCGGAATAGAATTGAATCATCATTGAATGGAATCG AGTAGAATCATTGAAATCGAATGGAATCATAGAATGG AATCCA AT	6933
TEE-320	AATGGAATCGAAAGGAATAGAATGGAATGGATCGTTA TGGAAAGATATCGAATGGAATGGAATTGACTCGAATG GAATG GACTGGAATGGAACG	6934
TEE-321	TAACGGAATAATCATCGAACAGAATCAAATGGAATCA TCATTGAATGGAATTGAATGGAATCTTCGAATAGACAT GAATG GACCATCATCG	6935
TEE-322	AACGGAATCAAACGGAATTATCGAATGGAATCGAATA GAATCATCGAACGGACTCGAATGGAATCATCTAATGG AATGGA ATGGAAG	6936

TEE-323	ATTGGAATGGAACGGAACAGAACGGAATGGAATGGAA TAGAATGGAATGGAATGGAATGGTATGGAATGGAATG GAATG GTACG	6937
TEE-324	AATCCACAAAGACAACAGAAGAAAAGACAACAGTAG ACAAGGATGTCAACCACATTTTGGAAGAGACAAGTAA TCAAAC ACATGGCA	6938
TEE-325	GAATCGAATGGAATCAACATCAAACGGAAAAAAACGG AATTATCGAATGGAATCGAAAAGAATCATCGAACGGA CTCGA ATGGAATCATCTAATGGAATGGAATGGAAGAATCCAT GG	6939
TEE-326	AATGGAATCGAATGGAATCATCATCAAATGGAATCTA ATGGAATCATTGAACGGAATTGGATGGAATCGTCAT	6940
TEE-327	CAACATCAAACGGAAAAAAACGGAATTATCGAATGGA ATCGAAGAGAATCATCGAATGGACC	6941
TEE-328	CACAACCAAAGCAATGAAAGAAAAGCACAGACTTATT GAAATGAAAGTACACACCACAGAATGGGAGCAGGCTC AAGCA AGC	6942
TEE-329	ATCAAAGGGAATCAAGCGGAATTATCGAATGGAATCG AAGAGAATCATCGAATGGACTCGAATGGAATCATGTG ATGGA ATGGAATGGAATAATCCACGGACT	6943
TEE-330	GGAATCGAATGGAATCAATATCAAACGGAGAAAAACG GAATTATCGAATGGAATCGAAGAGAATCATCGAATGG ACC	6944
TEE-331	AGGAATGGACACGAACGGAATGCAATCGAATGGAATG GAATCTAATAGAAAGGAATTGAATGAAATGGACTGG	6945
TEE-332	GGAAGGGAATCAAATGCAACAGAATGTAATGGAATGG AATGCAATGGAATGCAATGGAATGGAATGGAATGCAA TGGAA TGG	6946
TEE-333	AAATTGGATTGAATCGAATCGAATGGAAAAAATGAAA TCAAATGAAATTGAATGGAATCGAAATGAATGTAAAC AATGG AATCCAATGGAATCCAATGGAATCGAATCAAATGGTTT TGAGTGGCGTAAAATG	6947
TEE-334	AATGGAAGGGAATGGAATGGAATCGAATCGAATGGAA CAGAATTCAATGGAATGGAATGGAATGGAATGGAATC GAATG GAATGG	6948
TEE-335	GAAAAATCATTGAACGGAATCGAATGGAATCATCATC GGATGGAAACGAATGGAATCATCATCGAATGGAAATG AAAGG AGTCATC	6949
TEE-336	GGAATCGAATGGAATCAACATCAAACGGAGAAAAACG GAATTATCGAATGGAATCGAAGAGAATCATCGAATGG ACC	6950

TEE-337	AAAGAAATGTCACTGCGTATACACACACACGCACATA CACACACCATGGAATACTACTCAGCTATACAAAGGAA TGAAAT AATCCACAGCCAC	6951
TEE-338	GGAATCGAATGGAATCAATATCAAACGGAAAAAAACG GAATTATCGAATGGAATCGAAGAGAATCATCGAATGG ACC	6952
TEE-339	TGAACGGAATCGAATGGAATCATCATCGGATGGAAAC GAATGGAATCATCATCGAATGGAAATGAAAGGAGTCA TC	6953
TEE-340	GAATAGAACGAAATGGAATGGAATGGAATGGAATGGA AAGGAATGGAATGGAATGGAACG	6954
TEE-341	TGGAATTATCGTCGAATAGAATCGAATGGTATCAACAT CAAACGGAAAAAAACGGAATTATCGAATGGAATCGAA GAGA ATCATCGAACGGACTCGAATGGAATCATCTAATGGAA TGGAATGGAATAATCCATGG	6955
TEE-342	GACAAAAAGAATCATCATCGAATAGAATCAAATGGAA TCTTTGAATGGACTCAAAAGGAATATCGTCAAATGGA ATCAAA AGCCATCATCGAATGGACTGAAATGGAATTATCAAAT GGACTCG	6956
TEE-343	AACCAAACCAAGCAAACAAACAAACAGTAAAAACTCA ATAACAACCAACAAACAGGAAATACCAGGTAATTCAG ATTAT CTAGTTATGTGCCATAGT	6957
TEE-344	GAATGAATTGAATGCAAACATCGAATGGTCTCGAATG GAATCATCTTCAAATGGAATGGAATGGAATCATCGCAT AGAAT CGAATGGAATTATCAACGAATGGAATCGAATGGAATC ATCATCAGATGGAAATGAATGGAATCGTCAT	6958
TEE-345	TGGAATGGAATCAAATCGCATGGAATCGAATGGAATA GAAAAGAATCAAACAGAGTGGAATGGAATGGAATGG AATGGA ATCATGCCGAATGGAATG	6959
TEE-346	AAATGGAATAATGAAATGGAATCGAACGGAATCATCA TCAAAAGGAACCGAATGAAGTCATTGAATGGAATCAA AGGCA ATCATGGTCGAATGGAATCAAATGGAAACAGCATTGA ATAGAATTGAATGGAGTCATCACATGGAATCG	6960
TEE-347	GAATTAACCCGAATAGAATGGAATGGAATGGAATGGA ACAGAACGGAACGGAATGGAATGGAATGGAATGGAAT GGAAT G	6961
TEE-348	AAGATATACAAGCAGCCAACAAACATACGAAAGAATG CTCAACATCACTAATCCTCAGAGAAATTTAAATCAAAA CCACA ATGAGTTACAATCTCATACCAGTCAGAAT	6962
TEE-349	AGATAAGTGGATGAACAGATGGACAGATGGATGGATG GATGGATGGATGGATGGATGCCTGGAAGAAAGAAGAA	6963

	TGGAT AGTAAGCTGGGTATA	
TEE-350	AGAATTACAAACCACTGCTCAACAAAATAAAAGAGTA CACAAACAAATGGAAGAATATTCCATGCTTATGGATA GGAAGA ATCAATATTGTGAAAATGGCCATACT	6964
TEE-351	CATCGAATGGACTCGAATGGAATAATCATTGAACGGA ATCGAAGGGAATCATCATCGGATGGAAACGAATGGAA TCATCA TCGAATGGAAATG	6965
TEE-352	AAAGGAATCAAACGGAATTATCGAATGGAATCGAAAA GAATCATCGAACGGACTCGAATGGAATCATCTAATGG AATGG AATGGAAGAATCCATGGACTCGAATG	6966
TEE-353	GGATATAAACAAGAAAACAACTAATCACAACTCAATA TCAAAGTGCAATGATGGTGCAAAATGCAAGTATGGTG GGGAC AGAGAAAGGATGC	6967
TEE-354	AACATCAAACGGAAAAAAACGGAAATATCGAATGGAA TCGAAGAGAATCATCGAATGGACC	6968
TEE-355	TAAAATGGAATCGAATGGAATCAACATCAAATGGAAT CAAATGGAATCATTGAACGGAATTGAATGGAATCGTC AT	6969
TEE-356	AATCATCATCGAATGGAATCGAATGGTATCATTGAATG GAATCGAATGGAATCATCATCAGATGGAAATGAATGG AATCG TCAT	6970
TEE-357	CAATGCGTCAAGCTCAGACGTGCCTCACTACGGCAATG CGTCAAGCTCAGGCGTGCCTCACTAT	6971
TEE-358	TAAGCTGATAAGCAACTTTAGCAAAGTCTCAGGATAC AAAATCAATGTACAAAAATCACAAGCATTCTTATACAC CAACA ACAGACAGACGGAGAGCCAAA	6972
TEE-359	AATCAAAGAATTGAATCGAATGGAATCATCTAATGTA CTCGAATGGAATCACCAT	6973
TEE-360	ATGAACACGAATGTAATGCAATCCAATAGAATGGAAT CGAATGGCATGGAATATAAAGAAATGGAATCGAAGAG AATGG AAACAAATGGAATGGAATTGAATGGAATGGAATTG	6974
TEE-361	ATCAAACGGAATCAAACGGAATTATCGAATGGAATCG AAGAGAATCATCGAACGGACTCGAATGGAATCATCTA ATGGAA TGGGATGG	6975
TEE-362	AATGGAAAGGAATCAAATGGAATATAATGGAATGCAA TGGACTCGAATGGAATGGAATGGAATGGACCCAAATG GAATG GAATGGAATGGAATG	6976
TEE-363	GGAATACAACGGAATGGAATCGAAAAAAATGGAAAG GAATGAAATGAATGGAATGGAATGGAATGGAATGGAT GGGAA	6977

	TGGAATGGAATGG	
TEE-364	GAATCAAGCGGAATTATCGAATGGAATCGAAGAGAAT CATCGAAAGGACTCGAATGGAATCATCTAATGGAATG GAATG GAATAATACACGGACC	6978
TEE-365	AAGATAACCTGTGCCCAGGAGAAAAACAATCAATGGC AACAAAAGCAGAAACAACACAAATGATACAATTAGCA GACAG AAACATTGAGATTGCTATT	6979
TEE-366	AATGGACTCCAATGGAATAATCATTGAACGGAATCTA ATGGAATCATCATCGGATGGAAATGAGTGGAATCATC ATCGAA TGGAATCG	6980
TEE-367	AATCTATAAACGTAATCCATCACATAAACAGGACCAA AGAGAAAAACCGCATGATTATCTCAAGAATGCAGAAA AGGCC	6981
TEE-368	TAATTGATTCGAAATTAATGGAATTGAATGGAATGCAA TCAAATGGAATGGAATGTAATGCAATGGAATGTAATA GAATG GAAAGCAATGGAATG	6982
TEE-369	AAAGGAATGGACTTGAACAAAATGAAATCGAACGATA GGAATCGTACAGAACGGAAAGAAATGGAACGGAATG GAATG	6983
TEE-370	TGAGCAGGGAACAATGCGGATAAATTTCACAAATACA ATGTTGAGCAAAAGAAAGACACAAAAGAATACACACA TACAC ACCATATGGGCTAGG	6984
TEE-371	AATGGAATCGAACGGAATCATCATCAAACGGAACCGA ATGGAATCATTGAATGGAATCAAAGGCAATCATGGTC GAATG	6985
TEE-372	AATGGAATGGAATGTACAAGAAAGGAATGGAATGAAA CCGAATGGAATGGAATGGACGCAAAATGAATGGAATG GAAGT CAATGG	6986
TEE-373	AACGGAAAAAAACGGAATTATCGAATGGAATCGAAGA GAATCATCGAATGGACC	6987
TEE-374	GGAATAATCATTGAACGGAATCGAATGGAATCATCAT CGGATGGAAACGAATGGAATCATCATCGAATGGAAAT GAAAG GAGTCATC	6988
TEE-375	GGAACGAAATCGAATGGAACGGAATAGAATAGACTCG AATGTAATGGATTGCTATGTAATTGATTCGAATGGAAT GGAAT CG	6989
TEE-376	TGAAAGGAATAGACTGGAACAAAATGAAATCGAATGG TAGGAATCATACAGAACAGAAAGAAATGGAACGGAAT GGAAT G	6990
TEE-377	AACCCGAATAGAATGGAATGGAATGGAATGGAACGGA ACGGAATGGAATGGAATGGATTGGAATGGAATGGAAT	6991

	G	
TEE-378	AAAGAGAATCAAATGGAATTGAATCGAATGGAATCGA ATGGATTGGAAAGGAATAGAATGGAATGGAATGGAAT GGAAT GGAATGGAATG	6992
TEE-379	AATGGAATCATCAGTAATGGAATGGAAAGGAATGGAA AGGACTGGAATGGAATGGAATGGAATGGAATGG	6993
TEE-380	GGAACAAAATGAAATCGAACGGTAGGAATCGTACAGA ACGGAAAGAAATGGAACGGAATGGAATGCACTCAAAT GGAAA GGAGTCCAATGGAATCGAAAGGAATAGAATGGAATGG	6994
TEE-381	AGAATGAGATCAAGCAGTATAATAAAGGAAGAAGTAG CAAAATTACAACAGAGCAGTGAAATGGATATGCTTTCT GGCA ATAATTGTGAAAGGTCTGGTAATGAGAAAGTAGCAAC AGCTAGTGGCTGCCAC	6995
TEE-382	AACAAATGGAATCAACATCGAATGGAATCGAATGGAA ACACCATCGAATTGAAACGAATGGAATTATCATGAAA TTGAAA TGGATGGACTCATCATCG	6996
TEE-383	TAACATGCAGCATGCACACACGAATACACAACACACA AACATGTATGCACGCACACGTGAATACACAACACACA CAAACA TGCATGCATGCATACATGAATACACAGCACACAAATA TCCAGCAT	6997
TEE-384	GAATGGAATCAACATCAAACGGAAAAAAAACGGAATT ATCGAATGGAATCGAATAGAATCATCGAATGGACC	6998
TEE-385	AATCGAATGAAATGGAGTCAAAAGGAATGGAATCGAA TGGCAAGAAATCGAATGTAATGGAATCGCAAGGAATT GATGT GAACGGAACGGAATGGAAT	6999
TEE-386	AATGGAATTGAACGGAAACATCAGCGAATGGAATCGA AAGGAATCATCATGGAATAGATTCGAATGGAATGGAA AGGAA TGGAATGGAATG	7000
TEE-387	ATGGAATCAACATCAAACAGAATCAAACGGAATTATC GAATGGAATCGAAGACAATCATCGAATGGACTCGAAT GGAATC ATCTAATGGAATGGAATGGAAGAATCCATGGTCTCGA ATGCAATCATCATCG	7001
TEE-388	GAATAATCATTGAACGGAATCGAATGGAATCATCTTCG GATGGAAACGAATGGAATCATCATCGAATGGAAATGA AAGGA GTCATC	7002
TEE-389	AATGGACTCGAATGGAATAATCATTGAACGGAATCGA ATGGAATCATCATCGGATGGAAATGAGTGGAATCATC ATCGAA TGGAATCG	7003
TEE-390	AAATGAAATCGAACGGTAGGAATCGTACAGAACGGAA AGAAATGGAACGGAATGGAATGCAATCGAATGGAAAG	7004

	GAGTC CAATGGAAGGGAATCGAAT	
TEE-391	TACCAAACATTTAAAGAACAAATATCAATCCTACGCA AACCATTCTGAAACACAGAGATGGAGGATATACAGCG AAACTC ATTCTACATGGCC	7005
TEE-392	TATTGGAATGGAATGGAATGGAGTCGAATGGAACGGA ATGCACTCGAATGGAAGGCAATGCAATGGAATGCACT CAACA GGAATAGAATGGAATGGAATGGAATGG	7006
TEE-393	GGAATTTAATAGAATGTACCCGAATGGAACGGAATGG AATGGAATTGTATGGCATGGAATGGAA	7007
TEE-394	GCAATCCAATAGAATGGAATCGAATGGCATGGAATAT AAAGAAATGGAATCGAAGAGAATGGAGACAAATGGA ATGGAA TTGAATGGAATGGAATTG	7008
TEE-395	AATGGAATCGAATGGAATCATCATCAAATGGAATCTA ATGGAATCATTGAACGGAATTAAATGGAATCGTCATC GAATGA ATTCAATGCAATCAACGAATGGTCTCGAATGGAACCA C	7009
TEE-396	AATTGCAAAAGAAACACACATATACACATATAAAACT CAAGAAAGACAAAACTAACCTATGGTGATAGAAATCA GAAAA GTACAGTACATTGGTTGTCTTGGTGGG	7010
TEE-397	TGACATCATTATTATCAAGAAACATTCTTACCACTGTT ACCAACTTCCCAACACAGACTATGGAGAGAGAGATAA GACAGA ATAGCATT	701 1
TEE-398	AAAGAATTGAATTGAATAGAATCACCAATGAATTGAA TCGAATGGAATCGTCATCGAATGGAATCGAAGGGAAT CATTGG ATGGGCTCA	7012
TEE-399	ATCATCGAATGGAATCGAATGGAATCAATATCAAACG GAAAAAAACGGAATTATCGAATGGAATCGAATAGAAT CATCGA ATGGACC	7013
TEE-400	GAATGAAATCGTATAGAATCATCGAATGCAACTGAAT GGAATCATTAAATGGACTTGAAAGGAATTATTATGGA ATGGAA TTG	7014
TEE-401	TAAGCAACTTCAGCAAAGTCTCAGGATACAAAATCAA TGTGCAAAAATCTCAAGCATTCTTATACACGAACAACA GACAA ACAGAGAGCT	7015
TEE-402	ACTCAAAAGGAATTGATTCGAATGGAATAGAATGGCA AGGAATAGTATTGAATTGAATGGAATGGAATGGACCC AAATG	7016
TEE-403	GAATGGAATTTAAAGGAATAGAATGGAAGGAATCGGA TGGAATGGAATGGAATAGAATGGAGTCGAATGGAATA	7017

	GAATC GAATGGAATGGCATTG	
TEE-404	TGAGAAAATGATGGAAAAGAGGAATAAAACGAAACA AAACCACAGGAACACAGGTGCATGTGAATGTGCACAG ACAAA GATACAGGGCGGACTGGGAAGGAAGTTTCTGCACCAG AATTTGGGG	7018
TEE-405	AACAAAAAATGAGTCAAGCCTTAAATAAAATCAGAGC CAAAAAAGAAGACATTACATCTGATAAGACAAAAATT CAAAG GACCATC	7019
TEE-406	AACCCAGTGGAATTGAATTGAATGGAATTGAATGGAA TGGAAAGAATCAATCCGAGTCGAATGGAATGGTATGG AATGGA ATGGCATGGAATCAAC	7020
TEE-407	ATCAACATCAAACGGAAAAAAAACGGAATTATCGAAT GGAATCGAAGAGAATCATCGAATGGACC	7021
TEE-408	AAGGAATGGAATGGTACGGAATAGAATGGAATGGAAC GAATTGTAATGGAATGGAATTTAATGGAACGGAATGG AATGG AATGGAATCAACG	7022
TEE-409	AACGGAATGGAAAGCAATTTAATCAAATGCAATACAG TGGAATTGAAGGGAATGGAATGGAATGGC	7023
TEE-410	AATCGAATGGAACGGAATAGAATAGACTCGAATGTAA TGGATTGCTATGTAATTGATTCGAATGGAATGGAATCG AATGG AATGCAATCCAATGGAATGGAATGCAATGCAATGGAA TGGAATCGAACGGAATGCAGTGGAAGGGAATGG	7024
TEE-41 1	TAGCAACATTTTAGTAACATGATAGAAACAAAACAGC AACATAGCAATGCAATAGTAACACAACAGCAACATCA TAACAT GGCAGCA	7025
TEE-412	AATGGAATCGAAGAGAATGGAAACAAATGGAATGGA ATTGAATGGAATGGAATTGAATGGAATGGGAAGGAAT GGAGTG	7026
TEE-413	AGCAAACAAGTGAATAAACAAGCAAACAAGTGAACA AGCAAACAAGTGAATAAACAAGCAAACAAGTGAACA AGCAAA CAAGTGAATAAACAAGCAAACAAGTGAACAAGGAAA CAAGTGAATAAACAAAGGCTCT	7027
TEE-414	AATGGAATCAACACGAGTGCAATTGAATGGAATCGAA TGGAATGGAATGGAATGGAATGAATTCAACCCGAATG GAATG GAAAGGAATGGAATC	7028
TEE-415	GAATCGAATGGAATCAACATCAAACGGAAAAAAACGG AATTATCGAATGGAATCGAAGAGAATCATCGAATGGA CC	7029
TEE-416	AACACGAATGTAATGCAATCCAATAGAATGGAATCGA ATGGCATGGAATATAAAGAAATGGAATCGAAGAGAAT GGAAA	7030

	CAAACGGAATGGAATTGAATGGAATGGAATTGAATGG AATGGGAACGAATGGAGTGAAATTG	
TEE-417	GAATGGAACGGAATAGAACAGACTCGAATGTAATGGA TTGCTATGTAATTGATTCGAATGGAATGGAATCGAATG GAATG CAATCCAATGGAATGGAATGCAATGCAATGGAATGGA ATCGAATGGAATGCAGTGGAAGGGAATGG	7031
TEE-418	GAATCGAATGGAATCAATATCAAACGGAAAAAAACGG AATTATCGAATGGAATCGAAGAGAATCATCGAATGGA CC	7032
TEE-419	ATAAACATCAAACGGAATCAAACGGAATTATCGAATG GAATCGAAGAGAATAATCGAATGGACTCAAATGGAGT CATCTA ATGGAATGGTATGGAAGAATCCATGGACTCCAACGCA ATCATCAGCGAATGGAATC	7033
TEE-420	AAAAGAAAAGACAAAAGACACCAATTGCCAATACTGA AATGAAAAAACAGGTAATAACTATTGATCCCATGGAC ATTAA AATGATGTTGAAGGAACACCAC	7034
TEE-421	AATGTCAAGTGGAATCGAGTGGAATCATCGAAAGAAA TCGAATGGAATCGAAGGGAATCATTGGATGGGCTCAA AT	7035
TEE-422	ATCATCGAATGGAATAGAATGGTATCAACATCAAACG GAGAAAAACGGAATTATCGAATGGAATCGAAGAGAAT CTTCGA ACGGACC	7036
TEE-423	GAATGGAATCATCGCATAGAATCGGATGGAATTATCA TCGAATGGAATCGAATGGTATCAACATCAAACGGAAA AAAACG GAATTATCGAATGGAATCGAATTGAATCATCGAACGG ACCCG	7037
TEE-424	AATGGACTCGAATGGAATAATCATTGAACGGAATCGA ATGGAATCATCATCGGATGGAAATGAATGGAATAATC CATGGA CTCGAATGCAATCATCATCGAATGGAATCGAATGGAA TCATCGAATGGACTCG	7038
TEE-425	AATGCAATCATCAACTGGCTTCGAATGGAATCATCAAG AATGGAATCGAATGGAATCATCGAATGGACTC	7039
TEE-426	AAGAGACCAATAAGGAATAAGTAAGCAACAAGAGGA AGGAGAAAAGGGCAAGAGAGATGACCAGAGTT	7040
TEE-427	TGGAATCATCATAAAATGGAATCGAATGGAATCAACA TCAAATGGAATCAAATGGAATCATTGAACGGAATTGA ATGGAA TCGTCAT	7041
TEE-428	GGAATCATCGCATAGAATCGAATGGAATTATCATCGA ATGGAATCGAATGGAATCAACATCAAACGAAAAAAAA CCGGA ATTATCGAATGGAATCGAAGAGAATCATCGAACGGAC C	7042
TEE-429	AAATCATCATCGAATGGGATCGAATGGTATCCTTGAAT	7043

	GGAATCGAATGGAATCATCATCAGATGGAAATGAATG GAATC GTCAT	
TEE-430	GGAATGTAATAGAACGGAAAGCAATGGAATGGAACGC ACTGGATTCGAGTGCAATGGAATCTATTGGAATGGAAT CGAAT GGAATGGTTTGGCATGGAATGGAC	7044
TEE-431	AAACAATGGAAGATAATGGAAAGATATCGAATGGAAT AGAATGGAATGGAATGGACTCAAATGGAATGGACTTT AATGG AATGG	7045
TEE-432	GGAACGAAATCGAATGGAACGGAATAGAATAGACTCG AATGTAATGGATTGCTATGTAATTGATTCGAATGGAAT GGAAT CGAATGGAATGCAATCCAATGGAATGGAATGCAATGC AATGAATGGAATGGAATGGAATGGAATGGAA	7046
TEE-433	AAACCGAATGGAATGGAATGGACGCAAAATGAATGGA ATGGAAGTCAATGGACTCGAAATGAATGGAATGGAAT GGAAT GGAATG	7047
TEE-434	GGAATCGAATGGAATCAACATCAAACGGAAAAAAACA GAATTATCGTATGGAATCGAATAGAATCATCGAATGG ACC	7048
TEE-435	CAACCCGAGTGGAATAAAATGGAATGGAATGGAATGA AATGGAATGGATCGGAATGGAATCCAATGGAATCAAC TGGAA TGGAATGGAATGGAATG	7049
TEE-436	TATCATCGAATGGAATCGAATGGAATCAACATCAAAC GGAAAAAAACGGAATTATCGAATGGAATCGAAGAGAA TCATC GAATGGACC	7050
TEE-437	CGGAATAATCATTGAACGGAATCGAATGGAATCATCA TCGGATGGAAACGAATGGAATCATCATCGAATGGAAA TGAAAG GAGTCATC	7051
TEE-438	CAACACACAGAGATTAAAACAAACAAACAAACAATCC AGCCCTGACATTTATGAGTTTACAGACTGGTGGAGAGG CAGAG AAG	7052
TEE-439	CACTACAAACCACGCTCAAGGCAATAAAAGAACACAA ACAAATGGAAAAACATTCCATGCTCATGGATGGG	7053
TEE-440	AATCGAATGGAATTAACATCAAACGGAAAAAAACGGA ATTATCGAATGGAATCGAAGAGAATCATCGAATGGAC C	7054
TEE-441	TGGAAAAGAATCAAATTGAATGGCATCGAACGGAATG GGATGGAATGGAATAGACCCAGATGTAATGGACTCGA ATGGA ATG	7055
TEE-442	GACTAATATTCAGAATATACAAGGAACTCAAACAACT CAACAGTAGAAAAAAAAACCTGAATAGACATTTCTCA	7056

	AAAGAA GACATACAAATGGCC	
TEE-443	GGTCCATTCGATGATTCTCTTCGATTCCATTCGATAATT CCGTTTTTTCCCGTTTGATGTTGATTCC	7057
TEE-444	GGAACGAAATCGAATGGAACGGAATAGAATAGACTCG AATGTAATGGATTGCTATGTAATTGATTCGAATGGAAT GGAAT CGAATGGAATGCAATCCAATGGAATGGAATGCAATGC AATGAATGGAATGGAATGGAATGGAATGGA	7058
TEE-445	AGCAACTTCAGTAAAGTGTCAGGATACAAAATCAATG TGCAAAAATCACAAGCATTCTTATACATCAATAACAGA CAAAC AGAGAGCCAAA	7059
TEE-446	GAATAATCATTGAACGGAATCGAATGGAATCATCATC GGATGGAAACGAATGGAATCATCATCGAATGGAAATG AAAGG AGTCATC	7060
TEE-447	TAATCATCTTCGAATTGAAAACAAAGCAATCATTAAAT GTACTCTAACGGAATCATCGAATGGACC	7061
TEE-448	GGAATCGAATGGAATCAACATCAAACGGAAAAAAACG GAATTATCGAATGGAATCGAAGAGAATCATCGAATGG ACC	7062
TEE-449	AGAGAAAAGATGATCATGTAACCATTGAAAAGACAAT GTACAAAACTAATACTAATCACACAGGACCAGAAAGC AATTTA GACCAT	7063
TEE-450	AATGGAATCGAATGGAATCAACATCAAACGGAAAAAA CGGAATTATCGAATGGAATCAAAGAGAATCATCGAAT GGACC	7064
TEE-451	AATGGAATTATCATCGAATGGAATCGAATGGAATCAA CATCAAACGGAAAAAAACGGAATTATCGAATGGAATC GAAGA GAATCATCGAATGGACC	7065
TEE-452	GTCAACACAGGACCAACATAGGACCAACACAGGGTCA ACACAGGACCAACATAGGACCAACACAGGGTCAACAC AAGAC CAACATGGGACCAACACAGGGTCAACATAGGACCAAC ATGGGACCAACACAGGGTCAACACAGGACCAAC	7066
TEE-453	GAATCAACTCGATTGCAATCGAATGGAATGGAATGGT ATTAACAGAATAGAATGGAATGGAATGGAATGGAACG GAACG	7067
TEE-454	ACTCGAATGCAATCAACATCAAACGGAATCAAACGGA ATTATCGAATGGAATCGAAGAGAATCATCGAACGGAC TCGAAT GGAATCATCTAATGGAATGGAATGG	7068
TEE-455	AATGGAATGGAATAATCGACGGACCCGAATGCAATCA TCATCGTACAGAATCGAATGGAATCATCGAATGGACT GGAATG GAATGG	7069
TEE-456	AATACAAACCACTGCTCAACGAAATAAAAGAGGATAC	7070

	AAACAAATGGAAGAACATTCTATGCTCATGGGTAGGA TGAATT CATATCGTGAAAATGGCCATACTGCC	
TEE-457	AAACACGCAAACACACACACAAGCACACTACCACACA AGCGGACACACATGCAAACACGCGAACACACACACAT ATACA CACAAGCACATTACAAAACACAAGCAAACACCAGCAG ACACACAAACACACAAACATACATGG	7071
TEE-458	AATCGAACGGAATCAACATCAAACGGAAAAAAAACGG AATTATCGAATGGAATCGAAGAGAATCATCGAATGGA CC	7072
TEE-459	TAATTGATTCGAATGGAATGGAATAGAATGGAATTGA ATGGAATGGACCATAATGGATTGGACTTTAATAGAAA GGGCAT G	7073
TEE-460	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAATG TACAAAAGTCACAAGCATTCTTATACACCAACAAAAG ACAAAC AGAGAGCC	7074
TEE-461	ACATCAAACGGAAAAAAAAAACAAAACGGAATTATCG AATGGAATCGAAGAGAATCATCGAATGGACC	7075
TEE-462	GAAATTCCAATTAAAATGAAATCGACTTATCTTAACAA ATATAGCAATGCTGACAACACTTCTCCGGATATGGGTA CTGCT	7076
TEE-463	ACATCTCACTTTTAGTAATGAACAGATCATTCAGACAG AAAATTAGCAAAGAAACATCAGAGTTAAACTACACTC TAAAC CAAATGGACCTA	7077
TEE-464	GAAGAAAGCATTCATTCAAGACATCTAACTCGTTGATA TAATGCATACAGTTCAAAATGATTACACTATCATTACA TCTAG GGCTTTC	7078
TEE-465	ACACACACATTCAAAGCAGCAATATTTACAACAGCCA AAAGGTGGAAACAATTGAGCAATTG	7079
TEE-466	ATCATCGAATAGAATCGAATGGTATCAACACCAAACG GAAAAAAACGGAATTATCGAATGGAATCGAAGAGAAT CTTCGA ACGGACC	7080
TEE-467	ATCAACATCAAACGGAAAAAACGGAATTATCGAATGG AATCGAAGAGAATCATCGAACGGACC	7081
TEE-468	AATCGAAAGGAATGTCATCGAATGGAATGGACTCAAA TGGAATAGAATCGGATGGAATGGCATCGAATGGAATG GAATG GAATTGGATGGAC	7082
TEE-469	AACATGAACAGTGGAACAATCAGTGAACCAATACAAG GGTTAAATAAGCTAGCAATTAAAAGCTGTATCACTGGT CTAAA GATAGAAGATCAAGTAGAAAATCAGCGCAAGAGGAA AGATATACGAAAACTAATGGCC	7083
TEE-470	CGAATGGAATCATTATGGAATGGAATGAAATGGAATA	7084

	ATCAAATGGAATTGAATGGAATCATCGAATGGAATCG AACAAA ATCCTCTTTGAATGGAATAAGATGGAATCACCAAATGG AATTG	
TEE-471	AAGGGAATTGAATAGAATGAATCCGAATGGAATGGAA TGGAATGGAATGGAATGGAATGGAATGGAATGGAATG GAATG	7085
TEE-472	GAATGGAATCGAATCAAATTAAATCAAATGGAATGCA ATAGAAGGGAATACAATGGAATAGAATGGAATGGAAT GGAAT GGACT	7086
TEE-473	AAACGGAATCAAACGGAATTATCGAATGGAATCGAAG AGAATCATCGAACGGACTCGAATGGAATCATCTAATG GAATG GAATGGAAGAATCCATGGACT	7087
TEE-474	ATGGAATCAACATCAAACGGAAAAAAAAACGGAATTA TCGAATGGAATCGAAGAGAATCATCGAATGGACCAGA ATGGA ATCATCTAATGGAATGGAATGG	7088
TEE-475	AATGGAATCATCATCGAATGGAATCGAATGGAATCAT GGAATGGAATCAAATGGAATCAAATGGAATCGAATGG AATGG AATGGAATG	7089
TEE-476	AACGGAATCAAACGGAATTACCGAATGGAATCGAATA GAATCATCGAACGGACTCGAATGGAATCATCTAATGG AATGGA ATGGAAG	7090
TEE-477	AAACGGAATCAAACGGAATTATCGAATGGAATCGAAA AGAATCATCGAACGGACTCGAATGGAATCATCTAATG GAATG GAATGGAAGAATCCATGG	7091
TEE-478	GAATGATACGGAATACAATGGAATGGAACGAAATGAA ATGGAATGGAATGGAATGGAATGGAATGGAATGG	7092
TEE-479	ACAGCAAGAGAGAAATAAAACGACAAGAAAACTACA AAATGCCTATCAATAGTTACTTTAAATATCAGTGGACC AAATCA GTGAAACAAAAGACACAGAGTGGC	7093
TEE-480	AATGGACTCGAATGGATTAATCATTGAACGGAATCGA ATGGAATCATCATCGGATGGTAATGAATGGAATCATC ATCGAA TGGAATCGG	7094
TEE-481	GAATGGAATCGAAAGGAATGTCATCGAATGGAATGGA ATGGAACGGAATGGAATCGAATGGAATGGACTCGAAT GGAAT AGAATCGAATGCAATGGCATCG	7095
TEE-482	ATCGAATGGAATCAACATCAGACGGAAAAAAACGGAA TTATCAAATGGAATCGAAGAGAATCATCGAATGGACC	7096
TEE-483	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAATG TGCAAAAATCAAAAGCATTCTTATGCACCAATAACAG ACACAG	7097

	AGCCAAAT	
TEE-484	AATGGAATGGAACGCAATTGAATGGAATGGAATGGAA CGGAATCAACCTGAGTCAAATGGAATGGAATGGAATG GAATG	7098
TEE-485	GGAACGAAATCGAATGGAACGGAATAGAATAGACTCG AATGTCATGGATTGCTATGTAATTGATTGGAATGGAAT GGAAT CG	7099
TEE-486	TAGCAGGAAACAGCAAACTCAAATTAAGTAATTTCAA GAGCGTATCATCAATGAACTATTTTCAAAGATGTGGGC AAGAT	7100
TEE-487	GAATTGAAAGGAATGTATTGGAATAAAATGGAATCGA ATAGGTTGAAATACCATAGGTTCGAATTGAATGGAAT GGGAGG GACACCAATGGAATTG	7101
TEE-488	AAGCAACTTCAGCAAAGTCTCGGGATACAAAATCAAT GTGCAAAAATCACAAGCATTCTTATACACCACTAACAG ACAAA TGGAGAGTC	7102
TEE-489	GAATGGAATCAACATCAAACGGAAAAAAACGGAATTA TCGAATGGAATCGAAGAGAATCATCGAATGGACCAGA ATGGA ATCATCTAATGGAATGGAATGGAATAATCCATGG	7103
TEE-490	AAAAGCAATTGGACTGATTTTAAATATACGTGGCAAC AAGGATAAACTGCTAATGATGGGTTTGCAAATACAGA TCG	7104
TEE-491	AATGGAATCAACATCGAACGGAAAAAAACGGAATTAT CGAATGGAATCGAAGAGAATCATCGAATGGACC	7105
TEE-492	AAACGGAATTATCAAATGGAATCGAAGAGAATCATCG AACGGACTCGAATGGAATCATCTAATGGAATGGAATG GAAG	7106
TEE-493	TGCAAGATAACACATTTTAGTTGACACCATTGAAAACA GTTTTAACCAAGAATATTAGAACCAATGAAGCAGAGA AATCA AAAGGGTGGATGGAACTGCCAAAGGATG	7107
TEE-494	TAGAACAGAATTGAATGGAATGGCATCAAATGGAATG GAAACGAAAGGAATGGAATTGAATGGACTCAAATGTT ATGGA ATCAAAGGGAATGGACTC	7108
TEE-495	AAGAGAATCATCGAATGGAATCGAATGGAATCAACAT CAAACGGAAAAAAACGGAATTATCGAATGGAATCGAA GAGAA TCATCGAATGGACC	7109
TEE-496	ATCAACATCAAACGGAAAAAAACGGAATTATCGAATG GAATCGAAGAGAATCATCGAATGGACC	7110
TEE-497	GAATCAACATCAAACGGAAAAAAACCGAATTATCGAA TGGAATCGAAGAGAATCATCGAATGGACC	7111
TEE-498	ATCAACATCAAACGGAATCAAACGGAATTATCGAATG GAATCGAAGAGAATCATCAAATGGACTCGAATGGAAT CATCTA	7112

	ATGGAATGGAATGGAAGAATCCATGG	
TEE-499	ATCGAATGGAATCATTGAATGGAAAGGAATGGAATCA TCATGGAATGGAAACGAATGGAATCACTGAATGGACT CGAATG GGATCATCA	7113
TEE-500	ATTCAGCCTTTAAAAAAAGAAGACAGTCCTGTCATTTG TGACAATATGAATGAAACAGACATCACATTAAATGAA ATGAG CCAGGCGCAG	7114
TEE-501	GAATGAAATGAAATCAAATGGAATGTACATGAATGGA ATAGAAAAGAATGCATCTTTCTCGAACGGAAGTGCATT GAATG GAAAGGAATCTACTGGAATGGATTCGAATGGAATGGA ATGGGATGGAATGGTATGG	7115
TEE-502	AACATCAAACGGAATCAAACGGAATTATCGAATGGAA TCGAAGAGAATCATCGAACGGACTCGAATGGAATCAT CTAATG GAATGGAATGGAAGAATCCATGGACTCGAATGCAATC ATCATCGAATGAAATCGAATGGAATCATCGAATGGAC TCG	7116
TEE-503	ATGGAATTCAATGGAATGGACATGAATGGAATGGACT TCAATGGAATGGTATCAAATGGAATGGAATTCAGT	7117
TEE-504	AATGGAAAGGAATCGAATGGAAGGGAATGAAATTGAA TCAACAGGAATGGAAGGGAATAGAATAGACGGCAATG GAAT GGACTCG	7118
TEE-505	AGCAACTTCAGCAAAGTATCAGGATACAAAATCAATG TACAAAAATCCCAAGCATTCTTATACACCAACAACAG ACAAAC AGAGAGCC	7119
TEE-506	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCGATG TGCAAAAATCACAAGCATTCTTATACACCAACAACAG ATAAAC AGAGAGCC	7120
TEE-507	AACGGAAAAAAAACGGAATTATCGAATGGAATCGAAG AGAATCATCGAATGGACCAGAATGGAATCATCTAATG GAATG GAATGGAATAATCCATGGACTCGAATG	7121
TEE-508	GGAATCAAACGGAATTATCGAATGGAATCGAAGAGAA TCATAGAACGGACTCAAATGGAATCATCTAATGGAAT GGAAT GGGAGAATCCATGGACTCGAATG	7122
TEE-509	AATGGAATCAATATCAAACGGAAAAAAACGGAATTAT CGAATGGAATCGAAGAGAATCATCGAATGGACC	7123
TEE-510	AACGGAATCAAACGGAATTATCGAATGGAATCGAAAA GAATCATCGAACGGACTCGAATGGAATCATCTAATGG AATGG AATGGAAGAATCCATGG	7124
TEE-51 1	AAACGGAATTATCGAATGGAATCAAAGAGAATCATCG AATGGCCACGAATGGAATCATATAATGGAATGGAATG	7125

	GAATA ATCCATGGACC	
TEE-512	AATGGAATCGAATGGATTGATATCAAATGGAATGGAA TGGAAGGGAATGGAATGGAATGGAATTGAACCAAATG TAATG GATTTG	7126
TEE-513	TAAAAGACGGAACAGATAGAAAGCAGAAAGGAAAGG TGAATTGCATTACCACTATTCATACTGCCACACACATG ACATTA GGCCAAGTC	7127
TEE-514	AATGGAATCGAATGGAACAATCAAATGGACTCCAATG GAGTCATCTAATGGAATCGAGTGGAATCATCGAATGG ACTCG	7128
TEE-515	TAACACATAAACAAACACAGAGACAAAATCTCCGAGA TGTTAATCTGCTCCAGCAATACAGAACAATTTCTATTA CCAAC AGAATGCTTAATTTTTCTGCCT	7129
TEE-516	GGAATCGAATGGAATCAACATCAAACGGAAAAAAACG GAATTATCGAATGGAATCAAAGAGAATCATCGAATGG ACC	7130
TEE-517	AGAATGGAAAGGAATCGAAACGAAAGGAATGGAGAC AGATGGAATGGAATG	7131
TEE-518	GAATCATCATAAAATGGAATCGAATGGAATCAACATC AAATGGAATCAAATGGTCTCGAATGGAATCATCTTCAA ATGGA ATGGAATGG	7132
TEE-519	AACAACAATGACAAACAAACAACAACGACAAAGACAT TTATTTGGTTCACAAATCTCCAGGGTGTACAAGAAGCA TGGTG CCAGCATCTGCTCAGCTTCTGATGAGGGCTCTGGGAAG CTTTTACTC	7133
TEE-520	AACGGACTCGAACGGAATATAATGGAATGGAATGGAT TCGAAAGGAATGGAATGGAATGGACAGGAAAAGAATT GAATG GGATTGGAATGGAATCG	7134
TEE-521	AACATCAAACGAAATCAAACGGAATTATCAAATTGAA TCGAAGAGAATCATCGAATTGCCACGAATGCAATCAT CTAATG GTATGGAATGGAATAATCCATGGACCCAGATG	7135
TEE-522	AGAAATTAACAGCAAAAGAAGGATGCAGTGCAACTCA GGACAACACATACAATTCAAGCAACAAATGTATAGTG GCTGG GCACCAAGGATACAG	7136
TEE-523	GCAATAAAATCGACTCAGATAGAGAAGAATGCAATGG AATGGAATGGAATGGAATGGAATGGGATGGAATGGTA TGGAA TGG	7137
TEE-524	AATGGACTCGAATGAAATCATCATCAAACGGAATCGA ATGGAATCATTGAATGGAAAGGATGGGATCATCATGG AATGGA	7138

	AACGAATGGAATCACTG	
TEE-525	CCACATAAAACAAAACTACAAGACAATGATAAAGTTC ACAACATTAACACAATCAGTAATGGAAAAGCCTAGTC AATGGC AG	7139
TEE-526	TGGAATGGAATGGAATGGAATCAAATCGCATGGTAAT GAATCAAATGGAATCAAATCGAATGGAAATAATGGAA TCGAA GGGAAACGAATGGAATCGAATTGCACTGATTCTACTG ACTTCGAGGAAAATGAAATGAAATGCGGTGAAGTGGA ATGG	7140
TEE-527	GAATGTTATGAAATCAACTCGAACGGAATGCAATAGA ATGGAATGGAATGGAATGGAATGGAATGGAATGG	7141
TEE-528	AATGGAATCATTGAATGGAATGGAATGGAATCATCAA AGAAAGGAATCGAAGGGAATCATCGAATGGAATCAAA CGGAA TCATCGAATGGAATGGAATGGAATG	7142
TEE-529	GGAATCAACATCAAACGGAAAAAAAACGGAATTATCG AATGGAATCGAAGAGAATCATCGAATGGACC	7143
TEE-530	GGAATAATCATCATCAAACAGAACCAAATGGAATCAT TGAATGGAATCAAAGGCAATCATGGTCGAATG	7144
TEE-531	GCATAGAATCGAATGGAATTATCATTGAATGGAATCG AATGGAATCAACATCAAACGGAAAAAAACGGAATTAT CGAATG GAATCGAAGAGAATCATCGAATGGACCC	7145
TEE-532	AATGGAATCGAAGAGAATCATCGAACGGACTCGAATG GAATCATCTAATGGAATGGAATGGAATAATCCATGGA CCCGAA TG	7146
TEE-533	AAATGAATCGAATGGAATTGAATGGAATCAAATAGAA CAAATGGAATCGAAATGAATCAAATGGAATCGAATCG AATGG AATTGAATGGCATGGAATTG	7147
TEE-534	AGTTAATCCGAATAGAATGGAATGGAATGCAATGGAA CGGAATGGAACGGAATGGAATGGAATGGAATGGAATG GAATG	7148
TEE-535	ATCACAATCACACAACACATTGCACATGCATAACATGC ACTCACAATACACACACAACACATACACAACACACAT GCAAT ACAACACAAAACGCAACACAACATATACACAACACAC AGCACACACATGCC	7149
TEE-536	AAAGACTTAAACGTTAGACCTAAAACCATAAAAACCC TAGAGGAAAACCTAGGCATTACCATTCAGGACTTAGG CATGGG CAAGGAC	7150
TEE-537	AAAGTCCAAAGATGAACAAAATATCCAGAAGGAAAAC AAATGCACTTGGGGAGTGGGAAAGAAAACCAAGACTG AGCAA TGCGTCAAGCTCAGACGTGCCTCACTACG	7151
TEE-538	AAACGGAATCAAACGGAATTATCGAATGGAGTCGAAA	7152

	AGAATCATCGAACGGACTCGAATGGAATCATCTAATG GAATG GAATGGAAGAATCCATGG	
TEE-539	AATTGATTCGAAATTAATGGAATTGAATGGAATGCAAT CAAATGGAATGGAATGTAATGCAATGGAATGTAATAG AATGG AAAGCAATGGAATG	7153
TEE-540	TACAGAACACATGACTCAACAACAGCAGAAAGCATAT TCTTTTCAAATGCACATGAAACATTATCATGATGGACC AAAT	7154
TEE-541	GGAACAAAATGAAATCGAACGGTAGGAATCATACAGA ACAGAAAGAAATGGAACGGAATGGAATG	7155
TEE-542	AACGGAAAAAACGGAATTATCGAATGGAATCGAAGAG AATCATCGAATGGAATCGAATGGAGTCATCG	7156
TEE-543	AATCGAACGGAATCAACATCAAACGGAAAAAAACGGA ATTATCGAATGGAATCGAAGAGAATCATCGAATGGAC C	7157
TEE-544	AGAATGGAATGCAATAGAATGGAATGCAATGGAATGG AGTCATCCGTAATGGAATGGAAAGGAATGCAATGGAA TGGAA TGGAATGG	7158
TEE-545	ATGGAATCAACATCAAACGGAATCAAACGGAATTATC GAATGGAATCGAAGAGAATCATCGAACGGATTCGAAT GGAATC ATCTAATGGAATGGAATGGAAGAATCCATGGACTCGA ATGCAATCATCAGCGAATGGAATCGAATGGAATCATC GAATGG ACTCG	7159
TEE-546	GGAATAAAACGGACTCAATAGTAATGGATTGCAATGT AATTGATTCGATTTCGAATGGAATCGCATGGAATGTAA TGGAA TGGAATGGAATGGAAGGC	7160
TEE-547	AATGGAATCAACATCAAACGGAAAAAAACGGAATTAT CGTATGGAATCGAAAAGAATTATCGAATGGACC	7161
TEE-548	TCAAACGGAAAAAAACGGAATTATCGAATGGAATCGA AGAGAATCATCGAATGGACC	7162
TEE-549	ACATCAAACGGAATCAAACGGAATTATCGAATGGAAT CGAAAAGAATCATCGAACGGACTCGAATGGAATCATC TAATGG AATGGAATGGAAGAATCCATGGACTCGAATG	7163
TEE-550	TGGAATCGAATGGAATCAACATCAAACGGAAAAAAAC GGAATTATCGAATGGAATCGAAGAGAATCATCGAATG GACC	7164
TEE-551	AATGGAATCGAATGCAATCATCGAACGGAATCGAATG GCATCACCGAATGGAATGGAATGGAATGGAATGGAAT GG	7165
TEE-552	AGAATTGATTGAATCCAAGTGGAATTGAATGGAATGG AATGGATTAGAAAGGAATGGAATGGATTGGAATGGAT TGGAAT GGAAAGG	7166

TEE-553	AACTGCATCAACTAACAGGCAAAATAACCAGCTAATA TCATAATGACAGGATTAAATTCACAAATGACAATATTA ACCGT AAATGTAAATGGGCTA	7167
TEE-554	GTAAACAAACAATCAAGCAAGTAAGAACAGAAATAAC AGCATTTGGCTTTTGAGTTAATGACAAGAACACTCGGC ATGGG AGCCTGGGTGAGCAAATCACAGATCTTC	7168
TEE-555	AAAGGAATGGACTGGAACAAAATGAAATCGAACGGTA GGAATCGTACAGAACGGACAGAAATGGAACGGCATGG AATGC ACTCG	7169
TEE-556	GAATCAACCCGAGCGGAAAGGAATGGAATGGAATGGA ATCAACACGAATGGAATGGAACGGAATGGAATGGGAT GGGAT GAAATGGAATGG	7170
TEE-557	AAGAAATGGAATCGAAGAGAATGGAAACAAACGGAA TGGAATTGAATGGAATGGAATTGAATGGAATGGGA	7171
TEE-558	GACATGCAAACACAACACACAGCACACATGGAACATG CATCAGACATGCAAACACAACACACATACCACACATG GCATAT GCATCAGACGTGCCTCACTAC	7172
TEE-559	AAAGGAATGCACTCGAATGGAATGGACTTGAATGGAA TGTCTCCGAATGGAACAGACTCGTATGAAATGGAATC GAATGG AATGGAATCAAATGGAATTGATTTGAGTGAAATGGAA TCAAATGGAATGGCAACG	7173
TEE-560	GGAACAAAATGAAATCGAACGGTAGGAATCGTACAGA ACGGAAAGAAATGGAACGGAATGGAATGCACTCGAAT GGAAA GGAGTCCAAT	7174
TEE-561	AAATTGATTGAAATCATCATAAAATGGAATCGAAGGG AATCAACATCAAATGGAATCAAATGGAATCATTGAAC GGAATT GAATGGAATCGTCAT	7175
TEE-562	AGAATGGAAAGCAATAGAATGGAACGCACTGGATTCG AGTGCAATGGAATCAATTGGAATGGAATCGAATGGAA TGGAT TGGCA	7176
TEE-563	AACACCAAACGGAAAAAAACGGAATTATCGAATGGAA TCGAAGAGAATCTTCGAACGGACCCGAATGGGATCAT CTAAT GGAATGGAATGGAATAATCCATGG	7177
TEE-564	AATGGAGACTAATGTAATAGAATCAAATGGAATGGCA TCGAATGGAATGGACTGGAATGGAATGTGCATGAATG GAATGG AATCGAATGGATTG	7178
TEE-565	AAATCGAATGGAACGCAATAGAATAGACTCGAATGTA ATGGATTGCTATGTAATTGATTCGAATGGAATGGAATC GACTG	7179

	GAATGCAATCCAATGGAATGGAATGCAATGCAATGGA ATGGAATCGAACGGAATGCAGTGGAAGGGAATGG	
TEE-566	AATCAACAAGGAACTGAAACAAGTAAACAAGAAAAC AAATAACACCATAAAACATGGGCAAAGGACATAAACA GACATT TTTCAAAAAAGACATACAAATGGCCGAG	7180
TEE-567	AATGGAATCAACATCAAACGGAAAAAAACGGAATTAT CGAATGGAATCGAAGAGAATCATCGAATGGACCCAGG CTGGT CTTGAACTCC	7181
TEE-568	ATTGAATGGGCTAGAATGGAATCATCTTTGAACGGAAT CAAAGGGAATCATCATCGAATGGAATCGAATGGAAAT GTCAA CG	7182
TEE-569	AATGGACTCGAATGGAATCAACATCAAATGGAATCAA GCGGAATTATCGAATGAAATCGAAGAGAATCATCGAA TGGACT CGAAAGGAATCATCTAATGGAATGGAATGGAATAATC CATGGACTCGAATGCAATCATCATCG	7183
TEE-570	AAACGGAAAAAAACGGAATTATTGAATGGAATCGAAG AGAATCTTCGAACGGACCCGAATGGAATCATCTAATG GAATG GAATGGAATAATCCATGG	7184
TEE-571	ACTCGAGTGGAATTGACTGTAACAAAATGGAAAGTAA CGGATTGGAATCGAATGGAACGGAATGGAATGGAATG GACAT	7185
TEE-572	TACAAACTTTAAAAAATGATCAACAGATACACAGTTA GCAAGAAAGAATTGAGGGCAAAGAATATGCCAGACAA ACTCA AGAGGAAGATGATGGTAGAGATAGGTCACATTGGAGT GTCA	7186
TEE-573	AAATCAACAACAAACGGAAAAAAAAGGAATTATCGAA TGGAATCAAAGAGAATCATCGAATGGACC	7187
TEE-574	AACGGAATCAAACGGAATTATCGAATGGAATCGAAAA GAATCATCGAACGGACTCGAATGGAATCATCTAATGT AATGGA ATGGAAGAATCCATGGACTCGAATG	7188
TEE-575	AACGGAAAAAAACGGAATTATCGAATGGAATCGAAGA GAATCATCGAATGGACCAGAATGGAATCATCTAATGG AATGG AATGGAATAATCCATGGACTCGAATG	7189
TEE-576	CAACATCAAACGGAAAAAAACGGAATTATGGAATGGA ATCGAAGAGAATCATCGAATGGACCCGAATGGAATCA TCTGA AATATAATAGACTCGAAAGGAATG	7190
TEE-577	ATGGAATCGAATGGAATGGACTGGAATGGAATGGATT CGAATGGAATCGAATGGAACAATATGGAATGGTACCA AATG	7191
TEE-578	GAATGGAATCAACATCAAACGGAAAAAAACGGAATTA TCGAATGGAATCGAAGAGAATCATCGAATGGACC	7192

TEE-579	AAATGGACTCGAATGGAATCATCATAGAATGGAATCG AATGCAATGGAATGGAATCTTCCGGAATGGAATGGAA TGGAATGGAATGGAG	7193
TEE-580	GAATCATCATAAAATGGAATCGAATGGAATCAACATC AAATGGAATCAAATGGAATCATTGAACGGAATTGAAT GGAATCGTCAT	7194
TEE-581	ATCGAATGGAATCAACATCAAACGGAAAAAAACGGAA TTATCGAATGGAATCGAAGAGAATCATCGAATGGACC	7195
TEE-582	AGCAACTTCAGCAAAGTCTCAGGATACAAAATCAATG TACAAAAATCACAAGCATTCTTATACACCAATAACAG ACAAACAGAGAGCCAAAA	7196
TEE-583	AGAAACAGAAAACAGTCAAACCAATGGGCAATCCATA TCAGATGCAGTATTATGAACAGAAGTGTAAAGAATGC ACCAGGCACAATGGC	7197
TEE-584	GATTGGAACGAAATCGAATGGAACGGAATAGAATAGA CTCGAATGTAATGGATTGCTATGTAATTGATTCGAATG GAATGGAATCGAATGGAATGCAATCCAATGGAATGGA ATGCAATGCAATGGAATGG	7198
TEE-585	ATGGAATGGAATAATCAACGTACTCGAATGCAATCAT CATCGTATAGAATCGAATGGAATCATCGAATGGACTC GAATGGAATAATCATTGAACGGAGTCGAATGGAATCA TCATCGGATGGAAAC	7199
TEE-586	AAAGAAATCGAATGGAATCAGTGTCGAATGGAATGGA ATGGAATCGAAGAATTGAATTGAGTAGAATCGAAGGG AATCATTGGATGGGCTCAAAT	7200
TEE-587	AGAAAAGATAACTCGATTAACAAATGAACAAACACCT GAATACACAAGTCTCAAAAGAAGACATAAAAATGGCC AAC	7201
TEE-588	ATGGAATCAACATCAAACGGAATCACACGGAATTATC GAATGGAATCGAAAAGAATCATCGAACGGACTCGAAT GGAATCATCTAATGGAATGGAATGGAAG	7202
TEE-589	AATGGAATCAACATCAAACGGAATCAAGCGAAATTAT CGAATGGAATCGAAGAGAATCATCGAATGGACTCGAA TGGAATCATCTAATGGAATGGAATGGGAT	7203
TEE-590	AAACACAGTACAAATACTAATTCAAATCAAACTTACTC AAAGTCATAATCAAACATGCCAGACGGGCTGAGGGGC AGCATTA	7204
TEE-591	GGAATCGAGTGGAATCATCGAAAGAAATCGAATGGAA TCATTGTCGAATGGAATGGAATGGAATCAAAGAATGG AATCGAAGGGAATCATTGGATGGGCT	7205
TEE-592	AAAGAAAGACAGAGAACAAACGTAATTCAAGATGACT GTTTACATATCCAAGAACATTAGATGGTCAAAGACTTT AAGAAGGAATACATTCAAAGGCAAAAAGTCACTTACT GATTTTGGTGGAGTTTGCCACATGGAC	7206
TEE-593	GAAAGGAATCATCATTGAATGCAATCACATGGAATCA TCACAGAATGGAATCGTACGGAATCATCATCGAATGG AATTGAATGGAATCATCAATTGGACTCGAATGGAATC ATCAAATGGAATCGATTGGAAGTGTCAAATGGACTCG	7207
TEE-594	CAATCAGAGCGGACACAAACAAATTGCATGGGAAGAA TCAATATCGTGAAAATGGCC	7208

TEE-595	CAGCGCACCACAGCACACACAGTATACACATGACCCA CAATACACACAACACACAACACATTCACACACCAC	7209
TEE-596	GCAAACAGAATTCAACACTACATTAGAACGATCATTC ATCACGACCTAGTAGGATGTTTTTCCTGGGATGCAAGG ATGGTTCAACAT	7210
TEE-597	CAATCAAAACAGCAATGAGATACCATTTTACACCAATC AAAATGGCTACTAAAAAGTCAAAAGCAAATGCC	721 1
TEE-598	TGGAATAGAATGGAATCAATGTTAAGTGGAATCGAGT GGAATCATCGAAAGAAATCGAATGGAATCATTGTCGA ATGGTATGGAATGGAATCA	7212
TEE-599	AATGGAATGGAATCATCGCATAGAATGGAATGGAATT ATCATCGAATTGAATCGAATGGTATCAACATCAAACG GAAAAAAACGGAAATATCGAATGGAATCGAAGAGAAT CATCGAACGGACC	7213
TEE-600	GAAAAACAAAACAAAACAAACAAACAAACAATCAAA AAAGTGGTAGCAGAAACCAGAAAGTCCATGTATATAG CTAATTGGCCTGGTTGT	7214
TEE-601	AGACCTTTCTCAGAAGACACACAAATTGCCAACAGGT ATATGAAAAAATGTTCAATATCACTAATCATCAGGGCG ATGCC	7215
TEE-602	CATGGAATCGAATGGAATTATCATCGAATGGAATCGA ATGGTACCAACACCAAACGGAAAAAAACGGAATTATC GAATGGAATCGAAGAGAATCTTCGAACGGACC	7216
TEE-603	AGAGCAGAAACAAATGGAATTGAAATGAAGACAACA ATCAAAAGCATCAATGAAATGAAAAGTTGGGTTTTGG AAGAGAGAAACAAT	7217
TEE-604	ACACAAACACACACACACACACACACACACACACACA CACACACACACACACACACACACACACACATAC	7218
TEE-605	AACAAACAAATGAGATGATTTCAGATAGTGATAAACA CTATAACATAATTAATTCGTGCCAATCAGAGCATAACA GTGGTGTGGTGGCTGTGGAACAGATAGCAGAC	7219
TEE-606	AATGGAATCGAGTGGAATGGAAGGCAATGGAATAGAA TGGAATGGAATCGAAAGGAACGGAATGGAATGGAATG GAATG	7220
TEE-607	AGAAATGGAATCGGAGAGAATGGAAACAAATGGAAT GGAATTGAATGGAATGGAATTGAATGGAATGGGAACG	7221
TEE-608	AAGAGAACTGCAAAACACTGCTCAAAGAAATCAGAGA TGACAAAAACACATGGAAAAACGTTTCATGCTCATGG ATTGGAAGACTTA	7222
TEE-609	AATCAACACGAATAGAATGGAACGGAATGGAATGGAA TGGAATGGAATGGAATGGAGTGGAATGGAACAGAATG GAGTGGAAT	7223
TEE-610	AACATCAAACGAAATCAAACGGAATTATCAAATTGAA TCGAAGAGAATCATCGAATTGCCACGAATGCAACCAT CTAATGGTATGGAATGGAATAATCCATGGACCCAGAT G	7224
TEE-61 1	CGGAATTATCATCGAATGTAATCGAATGGAATCAACAT CAAACGGAAAAAAACGGAATTATCGAATGGAATCGAA GAGAATCATCGAATGGACC	7225
TEE-612	TGGACACACACGAACACACACCTACACACACGTGGAC	7226

	ACACACGGACACATGGACACACACGAACACATGGACA CACACACGGGGACACACACAGACACACACAGAGACAC ACACGGACACATGG	
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TEE-614	AAATGGAATGGAATGCACTTGAAAGGAATAGACTGGA ACAAAATGAAATCGAACGGTAGGAATCATACAGAACA GAAAGAAATGGAACGGAATGGAATG	7228
TEE-615	ACCACACACAAAATACACCACACACCACACACACACC ACACACTATACACACACCACACACCACACAC	7229
TEE-616	AAAGAAATAGAAGGGAGTTGAACAGAATCGAATGGA ATCGAATCAAATGGAATCGAATGGCATCAAATGGAAT CGAATGGAATGTGGTGAAGTGGATTGG	7230
TEE-617	GGAATCATCATAAAATGGAATCGAATGGAATCATCAT CAAATGGAATCAAATGGAATCATTGAACGGAATTGAA TGGAATCGTCAT	7231
TEE-618	AAAGATCAATGTACAAAAATCAGCAGCATTTCTATAA ACCAACAATGTCCAGGCTGAGAGAGAAATCAAGAAAA CAATTC	7232
TEE-619	TGGAATGGAATGGAATGAAATAAACACGAATAGAATG GAACGGAATGGAACGGAATGGAATGGAATGGAATGG AAAG	7233
TEE-620	TAATCAGCACAATCAACTGTAGTCACAAAACAAATAG TAACGCAATGATAAAGAAACAGAGAACTAGTTCAAAT AAACATGATAAGATGGGG	7234
TEE-621	AAGCGGAATTATCAAATGGAATCGAAGAGAATGGAAA CAAATGGAATGGAATTGAATGGAATGGAATTGAATGG AATG	7235
TEE-622	AATGGAATCAACATCAAACGGAAAAAAACGGAATTAT CGAATGGAATCGAAGAGAATCATCGAATGGACC	7236
TEE-623	ACTTGAATCGAATGGAAAGGAATTTAATGAACTTAAA TCGAATGGAATATAATGGTATGGAATGGACTCATGGA ATGGAATGGAAAGGAATC	7237
TEE-624	TGGAATCATCATCGAAAGCAAGCGAATGGAATCATCA AATGGAAACGAATGGAATCATCGAATGGACTCGGATG GAATTGTTGAATGGACT	7238
TEE-625	TGGAATCAACATCAAACGGAAAAAAACGGAATTATCG AATGGAATCGAAGAGAATCATCGAATGGACC	7239
TEE-626	TAAGTGAATTGAATAGAATCAATCTGAATGTAATGAA ATGGAATGGAACGGAATGGAATGGAATGGAATGGAAT GGAATGGAATGG	7240
TEE-627	AGGAAAATTTAATCAGCAGGAATAGAAACACACTTGA GAAATCCATGTGGAATGAAAAGAGAATGGCTGAGCAG CAACAGATTGTCAAAAAGGAAATC	7241
TEE-628	AACATCAAACGGAAAAAAAACGGAATTATCGAATGGA ATCGAAGAGAATCATCGAATGGACC	7242
TEE-629	TAATTGAGAATAAGCATTCCAGTGGAAAAAAAACTAA ACAATTTGTTGTAAAACATCCTTAAAAGCATCAGAAAG TTAATACAGCAATGAAGAATTACAGGACCAAATTAAG	7243

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TEE-630	TATCATCGAATGGAATCGAATGGAATCAACATCAAAC GGAAAAAAACGGAATTATCGAATTGAATCGAAGAGAA TCATCGAATGGACC	7244
TEE-631	AGCAAAACAAACACAATCTGTCGTTCATGGTACTACG ACATACTGGGAGAGATATTCAAATGATCACACAAAAC AACATG	7245
TEE-632	AAGGATTCGAATGGAATGAAAAAGAATTGAATGGAAT AGAACAGAATGGAATCAAATCGAATGAAATGGAGTGG AATAGAAAGGAATGGAATG	7246
TEE-633	AACGGAATCAAACGGAATTATCGAATGGAATCGAAGA GAATCATCGAACGGACTCGAATGGAATCATCTAATGG AATGGAATGGAAGAATCCATGGACTCGAATGCAATCA TCATCGAATGGAATCGAACGGAATCATCGAATGGCC	7247
TEE-634	AATCAACTAGATGTCAATGGAATGCAATGGAATAGAA TGGAATGGAATTAACACGAATAGAATGGAATGGAATG GAATGGAATGG	7248
TEE-635	AATGGACTCGAATGGAATAATCATTGAACGGAATCGA ATGGAATCATCATCGGATGGAAATGAATGGAATCATC ATCGCATGGAATCG	7249
TEE-636	GAATGGAATGATACGGAATAGAATGGAATGGAACGAA ATGGAATTGAAAGGAAAGGAATGGAATGGAATGGAAT GG	7250
TEE-637	AATCATCATCGAATGGAATCGAATGGTATCATTGAGTG GAATCGAATGGAATCATCATCAGATGGAAATGAATGG AATCGTCAT	7251
TEE-638	GAATCAAATCAATGGAATCAAATCAAATGGAATGGAA TGGAATTGTATGGAATGGAATGGCATGG	7252
TEE-639	TAATGCAGTCCAATAGAATGGAATCGAATGGCATGGA ATATAAAGAAATGGAATCGAAGAGAATGGGAACAAAT GGAATGGAATTGAGTGGAATGGAATTGAATGGAATGG GAACGAATGGAGTG	7253
TEE-640	AACATCAAACGGAAAAAAACGGAATTATCGAATGGAA TCGAAGAGAATCATCGAATGGACC	7254
TEE-641	ATCAAAAGGAACGGAATGGAATGGAATGGAATGGAAT GGAATGGAATGGAATGGAATGAAATCAACCCGAATGG AATGGATTGGCATAGAGTGGAATGG	7255
TEE-642	GCCAACAATCATATGAGAAAAAGCTCAACATCACTGA TCATTTCAGGAATGCAAATCAAAACCACAATGAGATA CTATCA	7256
TEE-643	AATCAAATGGAATGAAATCGAATGGAATTGAATCGAA TGGAATGCAATAGAATGTCTTCAAATGGAATCGAATG GAAATTGGTGAAGTGGACGGGAGTG	7257
TEE-644	TAATGGAATCAACATCAAACGGAAAAAAACGGAATTA TCGAATGCAATCGAAGAGAATCATCGAATGGACC	7258
TEE-645	AGCAACTTCAGCAAAGTCTCAGCATACAAAATCAATG TGCAAAAATCACACGCATTCCTATACACCAATAACAG ACAAACAGAGAGCC	7259
TEE-646	GAATCAAATGGAATGGACTGTAATGGAATGGATTCGA ATGGAATCGAATGGAGTGGACTCAAATGGAATG	7260

TEE-647	AACAAGTGGACGAAGGATATGAACAGACACTTCTCAA GACATTTATGCAGCCAACAGACACACGAAAAAATGCT CATCATCACTGGCCATCAG	7261
TEE-648	AAACGGAAAAAAACGGAATTATCGAATGGAATCGAAT AGAATCATCGAATGGACC	7262
TEE-649	TGGAACCGAACAAAGTCATCACCGAATGGAATTGAAA TGAATCATAATCGAATGGAATCAAATGGCATCTTCGAA TTGACTCGAATGCAATCATCCACTGGGCTT	7263
TEE-650	AACGGAATCACGCGGAATTATCGAATGGAATCGAAGA GAATCATCGAATGGACTCGAATGGAATCATCTAATGG AATGGAATGG	7264
TEE-651	GGAATCAACTCGATTGCAATGGAATGCAATGGAAAGG AATGGAATGCAATTAAAGCGAATAGAATGGAATGGAA TGGAATGGAACGGAATGGAATG	7265
TEE-652	AAAACAAACAACAACGACAAATCATGAGACCAGAGTT AAGAAACAATGAGACCAGGCTGGGTGTGGTG	7266
TEE-653	AATCGAAAGGAATGCAATATTATTGAACAGAATCGAA AAGAATGGAATCAAATGGAATGGAACAGAGTGGAATG GACTGC	7267
TEE-654	AAGGAATCGAATGGAAGTGAATGAAATTGAATCAACA GGAATGGAAGGGAATAGAATAGACTGT AATGGAATGG ACTCG	7268
TEE-655	AACCCGAGTGCAATAGAATGGAATCGAATGGAATGGA ATGGAATGGAATGGAATGGAATGGAGTC	7269
TEE-656	GAATGGAATTGAAAGGAATGGAATGCAATGGAATGGA ATGGGATGGAATGGAATGCAATGGAATCAACTCGATT GCAATG	7270
TEE-657	GAAAAAAACGGAATTATCGAATTGAATCAAATAGAAT CATCGAACGGACCAAAATGGAATCATCTAATGGAATG GAATGGAATAATCCATGGACTCTAATG	7271
TEE-658	TGGAATCATCTAATGGAATGGAATGGAATAATCCATG GACTCGAATGCAATCATCATAAAATGGAATCGAATGG AATCAACATCAAATGGAATCAAATGGGATCATTGAAC GGAATTGAATGGAATCGTCAT	7272
TEE-659	GAAAAAAACGGAATTATCGAATTGAATCGAATAGAAT CATCGAACGGACCAGAATGGAATCATCTAATGGAATG GAATGGAATAATCCATGGACTCGAATG	7273
TEE-660	AACCACTGCTTAAGGAAATAAGAGAGAACACAAACAA ATGGAAAAACGTTCCATGCTCATGGATAGGAGAATCA ATATCGTGAAAATGGCC	7274
TEE-661	TATCGAATGGAATGGAAAGGAGTGGAGTAGACTCGAA TAGAATGGACTGGAATGAAATAGATTCGAATGGAATG GAATGGAATGAAGTGGACTCG	7275
TEE-662	GTATCAACATCAAACGGAAAAAAACGGAATTATCGAA TGGAATCATCTAATGGAATGGAATGGAATAATCCATG GACTCGAATG	7276
TEE-663	TAAATGGAGACATCATTGAATACAATTGAATGGAATC ATCACATGGAATCGAATGGAATCATCGTAAATGCAAT CAAGTGGAATCAT	7277
TEE-664	GAATGGAATTGAAAGGTATCAACACCAAACGGAAAAA	7278

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TEE-665	AGCAATTTCAGCAAAGTCTCAGGATACAAAATCAATG TACAAATTCACAAGCATTCTTATGGACCAACAACAG	7279
TEE-666	GGAATCGAATGGCATCAACATCAAACGGAAAAAAACG GAATTATCGAATGGAATCGAATGGAATCATC	7280
TEE-667	AAACAAAACACAGAAATGCAAAGACAAAACATAAAA CGCAGCCATAAAGGACATATTTTAGATAACTGGGGAA ATTTGTATGGGCTGTGT	7281
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TEE-669	AATCGAATGGAATCAGCATCAAACGGAAAAAAACGGA ATTATCGAATGGAATCGAAGAGAATCATCGAATGGAC C	7283
TEE-670	AAACGGAATTATAGAATGGACTGGAAGAGAATCATCG AACGGACTAGAATGGAATCATCTAATCGAATGGAATG GAACAATCCATGGTCTAGCA	7284
TEE-671	TGAACAGAGAATTGGACAAAACGCACAAAGTAAAGAA AAAGAATGAAGCAACAAAAGCAGAGATTTATTGAAAA CAAAAGTACACACCACACAGGGTGGGAGTGG	7285
TEE-672	ATCATAACGACAAGAACAAATTCACACACAACAATAT TAACTTCAAATCCAAATGGGTTAAATGCTCCAATTAAA GGATGCAGACGGGCAAATTGGATA	7286
TEE-673	ATCATAACGACAAGAACAAATTCACACACAACAATAT TCACTTCAAATCCAAATGGGTTAAATGCTCCAATTAAA GGATGCAGACGGGCAAATTGGATA	7287
TEE-674	GAATGGAATCGAATGGATTGATATCAACTGGAATGGA ATGGAAGGGAATGGAATGGAATGGAATTGAACCAAAT GTAATGACTTGAATGGAATG	7288
TEE-675	GAATCAACATCAAACGGAAAAAAACGGAATTATCGAA TGGAATCGAAGAGAATCATCGAATGGACC	7289
TEE-676	GGAATCAACATCAAACGGAAAAAAACGGAATTATCGA ATGGAATCGAAGAGAATCATCGAATGGACC	7290
TEE-677	ATGGAATCAACATCAAACGGAATCAAACGGAATTATC GAATGGAATCAAAGAGAATCATCGAACGGACTCGAAT GGAATCATCTAATGGAATGGAATGGAAGAATCCATGG ACTCGAATGCAATCATCATCGAAT	7291
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TEE-679	AAAACACCTAGGAATACAGATAACAAGGGACATTAAC TACCTCTTAAAGAGAACTACAAACCACTGCTCAAGGA AATGAGAGAGGACACAAACACATGGAAAAACATTCCA TCCTCATGGATAGGAAGAATCAATATTGTGAAAATGG CC	7293
TEE-680	AACACGACTTTGAGAAGAGTAAGTGATTGTTAATTAA AGCAAGAGAATTATTGATGTATCACAGTCATGAGAAA TATTGGAAGGAATATGGTCCATAC	7294
TEE-681	ACACATATCAAACAAACAAAAGCAATTGACTATCTAG AAATGTCTGGGAAATGGCAAGATATTACA	7295

TEE-682	GGAATCATCATATAATGGAATCGAATGGAATCAACAT CAAATGGAATCAAATGGAATCATTGAACGGAATTGAA TGGAATCGTCAT	7296
TEE-683	AATGGAATCAACATCAAACGGAATCAAATGGAATTAT CGAATGGAATCGAAGAGAATCATCGAATTGTCACGAA TGGAATCATCTAATGGAATGGAATGGAATAATCCATG GCCCCTATGCAATGGACTCGAATGAAATCATCATCAAA CAGAATCGAATGGAATCATCTAATGGAATGGAATGGC ATAATCCATGGACTCGAATG	7297
TEE-684	TAAAATGAAACAAATATACAACACGAAGGTTATCACC AGAAATATGCCAAAACTTAAATATGAGAATAAGACAG TCTCAGGGGCCACAGAG	7298
TEE-685	AAAATACAGCGTTATGAAAAGAATGAACACACACACA CACACACACACACAGAAAATGT	7299
TEE-686	CAAACAAATAGGTACCAAACAAATAACAACATAAACC TGACAACACACTTATTTACAAGAGACATCCCTTATATG AAAGGGTACAGAAAAGTCGATGGTAAGATGATGGGGA AAGGTATACCAACCACTAGCAGAAGG	7300
TEE-687	TGGAATCGAATGGAATCAATATCAAACGGAAAAAAAC GGAATTATCGAATGGAATCGAAAAGAATCATCGAATG GGCCCGAATGGAATCATCT	7301
TEE-688	ACAAATGGAATCAACAACGAATGGAATCGAATGGAAA CGCCATCGAAAGGAAACGAATGGAATTATCATGAAAT TGAAATGGATG	7302
TEE-689	AATCAATAAATGTAAACCAGCATATAAACAGAACCAA CGACAAAAACCACATGATTATCTCAATAGATGCAGAA AAGGCC	7303
TEE-690	AAAATAAACGCAAATTAAAATCACAAGATACCAACAC ATTCCCACGGCTAAGTACGAAGAACAAGGGCGAATGG TCAGAATTAAGCTCAAACCT	7304
TEE-691	CAACATCAAACGGAATCAAACGGAATTATCGAATGGA ATCGAAGAGAATCATCGAATGGACTCGAATGGAATCA TCTAATGGAATGGAATGGAAG	7305
TEE-692	ACATCAAACGGAAAAAAACGGAATTATCGAATGGAAT CGAAGAGAATCATCGAATGGACC	7306
TEE-693	AATGGACTCGAATAGAATTGACTGGAATGGAATGGAC TCGAATGGAATGGAATGGAATGGAAGGGACTCG	7307
TEE-694	AAGAAAGACAGAGAACAAACGTAATTCAAGATGACTG ATTACATATCCAAGAACATTAGATGGTCAAAGACTTTA AGAAGGAATACATTCAAAGGCAAAACGTCACTTACTG ATTTTGGTGGAGTTTGCCACATGGAC	7308
TEE-695	GAATGGAATCGAATGGAATGAACATCAAACGGAAAAA AACGGAATTATCGAATGGAATCAAAGAGAATCATCGA ATGGACCCG	7309
TEE-696	ATGGACTCGAATGTAATAATCATTGAACGGAATCGAA TGGAATCATCATCGGATGGAAACGAATGGAATCATCA TCGAATGGAATCGAATGGGATC	7310
TEE-697	GAAATGGAATGGAAAGGAATAAAATCAAGTGAAATTG GATGGAATGGATTGGAATGGATTGGAATG	731 1
TEE-698	AAACGGAAAAAAAACGGAATTATCGAATGGAATCGAA	7312

	GAGAATCATCGAACGAACCAGAATGGAATCATCTAATGGAATGGAATGGAATAATCCATGG	
TEE-699	ATTAACCCGAATAGAATGGAATGGAATGGAATGGAACGGAACGGAATGGAATGGAATGGAATGGAATGGAATGGATCG	7313
TEE-700	AACATCAAACGGAAAAAAACGGAATTATCGTATGGAATCGAAGAGAATCATCGAATGGACC	7314
TEE-701	GAATAGAATTGAATCATCATTGAATGGAATCGAGTAGAATCATTGAAATCGAATGGAATCATCATCGAATGGAATTGGGTGGAATC	7315
TEE-702	CACCGAATAGAATCGAATGGAACAATCATCGAATGGACTCAAATGGAATTATCCTCAAATGGAATCGAATGGAATTATCG	7316
TEE-703	AATGCAATCGAATAGAATCATCGAATAGACTCGAATGGAATCATCGAATGGAATGGAATGGAACAGTC	7317
TEE-704	AAATCATCATCGAATGGAATCGAATGGTATCATTGAATGGAATCGAATGGAATCATCATCAGATGGAAATGAATGGAATCGTCAT	7318
TEE-705	GAATGGAATCGAAAGGAATAGAATGGAATGGATCGTTATGGAAAGACATCGAATGGAATGGAATTGACTCGAATGGAATGGACTGGAATGGAACG	7319

Example 46. *In Vitro* Expression of modified nucleic acids with miR-122

[001095] MicroRNA controls gene expression through the translational suppression and/or degradation of target messenger RNA. The expression of G-CSF mRNA and Factor IX mRNA with human or mouse alpha-globin 3' untranslated regions (UTRs) were down regulated in human primary hepatocytes using miR-122 sequences in the 3'UTR.

[001096] Primary human hepatocytes were seeded at a density of 350000 per well in 500 ul cell culture medium (InVitro GRO medium from Celsis, Chicago, IL).

[001097] G-CSF mRNA having a human alpha-globin 3'UTR (G-CSF Hs3 'UTR; mRNA sequence shown in SEQ ID NO: 7320; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a mouse alpha-globin 3'UTR (G-CSF Mm3'UTR; mRNA sequence shown in SEQ ID NO: 7321; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine. G-CSF mRNA containing a human 3'UTR having a miR-122 sequence in the 3'UTR (G-CSF Hs3'UTR miR-122; mRNA sequence shown in SEQ ID NO: 7322; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-122 seed sequence in the 3'UTR (G-CSF

Hs3'UTR miR-122 seed; mRNA sequence shown in SEQ ID NO: 7323; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF Hs3'UTR miR-122 seedless; mRNA sequence shown in SEQ ID NO: 7324; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine. G-CSF mRNA containing a mouse 3'UTR having a miR-122 sequence in the 3'UTR (G-CSF Mm3'UTR miR-122; mRNA sequence shown in SEQ ID NO: 7325; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-122 seed sequence in the 3'UTR (G-CSF Mm3'UTR miR-122 seed; mRNA sequence shown in SEQ ID NO: 7326; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF Mm3'UTR miR-122 seedless; mRNA sequence shown in SEQ ID NO: 7327; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine.

[001098] Factor IX mRNA having a human alpha-globin 3'UTR (Factor IX Hs3'UTR; mRNA sequence shown in SEQ ID NO: 7328; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a mouse alpha-globin 3'UTR (Factor IX Mm3'UTR; mRNA sequence shown in SEQ ID NO: 7329; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine. Factor IX mRNA containing a human 3'UTR having a miR-122 sequence in the 3'UTR (Factor IX Hs3'UTR miR-122; mRNA sequence shown in SEQ ID NO: 7330; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-122 seed sequence in the 3'UTR (Factor IX Hs3'UTR miR-122 seed; mRNA sequence shown in SEQ ID NO: 7331; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-122 sequence without the seed sequence in the 3'UTR (Factor IX Hs3'UTR miR-122 seedless; mRNA sequence shown in SEQ ID NO: 7332; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine. Factor IX mRNA containing a mouse 3'UTR having a miR-122 sequence in the 3'UTR (Factor IX Mm3'UTR miR-122;

mRNA sequence shown in SEQ ID NO: 7333; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-122 seed sequence in the 3'UTR (Factor IX Mm3'UTR miR-122 seed; mRNA sequence shown in SEQ ID NO: 7334; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-122 sequence without the seed sequence in the 3'UTR (Factor IX Mm3'UTR miR-122 seedless; mRNA sequence shown in SEQ ID NO: 7335; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine.

[001099] Each G-CSF or Factor IX mRNA sequence was tested at a concentration of 500 ng per well in 24 well plates. 24, 48 and 72 hours after transfection, the expression of protein was measured by ELISA. The protein levels for G-CSF are shown in Table 36 and the protein levels for Factor IX are shown in Table 37.

Table 36. G-CSF Protein Expression

Description	Protein Expression (ng/ml)		
	24 Hours	48 Hours	72 Hours
G-CSF Hs3'UTR	43.9	18.8	5.7
G-CSF Hs3'UTR miR-122	6.9	0.7	0.12
G-CSF Hs3'UTR miR-122 seed	48.5	25.6	8.2
G-CSF Hs3'UTR miR-122 seedless	31.7	11.7	3.4
G-CSF Mm3'UTR	84.9	100.4	21.3
G-CSF Mm3'UTR miR-122	24.0	3.03	0.8
G-CSF Mm3'UTR miR-122 seed	115.8	96.4	19.2
G-CSF Mm3'UTR miR-122 seedless	113.1	92.9	18.9

Table 37. Factor IX Protein Expression

Description	Protein Expression (ng/ml)		
	24 Hours	48 Hours	72 Hours
G-CSF Hs3'UTR	43.9	18.8	5.7
G-CSF Hs3'UTR miR-122	6.9	0.7	0.12
G-CSF Hs3'UTR miR-122 seed	48.5	25.6	8.2
G-CSF Hs3'UTR miR-122 seedless	31.7	11.7	3.4
G-CSF Mm3'UTR	84.9	100.4	21.3
G-CSF Mm3'UTR miR-122	24.0	3.03	0.8
G-CSF Mm3'UTR miR-122 seed	115.8	96.4	19.2
G-CSF Mm3'UTR miR-122 seedless	113.1	92.9	18.9

Example 47. *In Vitro* Expression of modified nucleic acid with mir-142 or miR-146 binding sites

[001100] HeLa and RAW264 cells were seeded at a density of 17000 and 80000 per well respectively, in 100 ul cell culture medium (DMEM+10%FBS).

[001101] G-CSF mRNA (G-CSF; mRNA sequence shown in SEQ ID NO: 6595; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) was fully modified with 5-methylcytidine and 1-methylpseudouridine.

[001102] G-CSF mRNA having a miR-142-3p sequence in the 3'UTR (G-CSF miR-142-3p; mRNA sequence shown in SEQ ID NO: 6634; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-142-3p seed sequence in the 3'UTR (G-CSF miR-142-3p seed; mRNA sequence shown in SEQ ID NO: 6636; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-142-3p sequence without the seed sequence in the 3'UTR (G-CSF miR-142-3p seedless; mRNA sequence shown in SEQ ID NO: 6638; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine.

[001103] G-CSF mRNA having a miR-142-5p sequence in the 3'UTR (G-CSF miR-142-5p; mRNA sequence shown in SEQ ID NO: 6628; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-142-5p seed sequence in the 3'UTR (G-CSF miR-142-5p seed; mRNA sequence shown in SEQ ID NO: 6630; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-142-5p sequence without the seed sequence in the 3'UTR (G-CSF miR-142-5p seedless; mRNA sequence shown in SEQ ID NO: 6632; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine.

[001104] G-CSF mRNA having a miR-146a sequence in the 3'UTR (G-CSF miR-146a; mRNA sequence shown in SEQ ID NO: 6640; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), or a miR-146a seed sequence in the 3'UTR (G-CSF miR-146a seed; mRNA sequence shown in SEQ ID NO: 6642; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a miR-146a sequence without the seed sequence in the 3'UTR (G-CSF miR-146a seedless; mRNA sequence shown in SEQ ID NO: 6644; polyA tail of approximately 140 nucleotides not

shown in sequence; 5'cap, Capl) were fully modified with 5-methylcytidine and 1-methylpseudouridine.

[001105] Each G-CSF mRNA sequence was tested at a concentration of 500 ng per well in 24 well plates for each cell type. 24 hours after transfection, the expression of protein was measured by ELISA and the protein levels are shown in Table 38. The G-CSF sequences with a miR-142-3p sequence in the 3'UTR down regulated G-CSF expression in RAW264 cells whereas the G-CSF sequences with a miR-142-5p or miR-146a sequence in the 3'UTR did not down regulate G-CSF expression.

Table 38. G-CSF Expression

Description	HeLa Cells	RAW264 Cells
	Protein Expression (ng/ml)	Protein Expression (ng/ml)
G-CSF	243.5	173.7
G-CSF miR-142-3p	309.1	67.6
G-CSF miR-142-3p seed	259.8	178.1
G-CSF miR-142-3p seedless	321.7	220.2
G-CSF miR-142-5p	291.8	223.3
G-CSF miR-142-5p seed	261.3	233.1
G-CSF miR-142-5p seedless	330.2	255.1
G-CSF miR-146a	272.6	125.2
G-CSF miR-146a seed	219.4	138.3
G-CSF miR-146a seedless	217.7	132.8

Example 48. Effect of Kozak sequence on expression of modified nucleic acids

[001106] HeLa cells were seeded at a density of 17000 per well in 100 ul cell culture medium (DMEM + 10%FBS). G-CSF mRNA having an IRES sequence and Kozak sequence (G-CSF IRES Kozak; mRNA sequence shown in SEQ ID NO: 7336; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), G-CSF mRNA having an IRES sequence but not a Kozak sequence (G-CSF IRES; mRNA sequence shown in SEQ ID NO: 7337; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl), G-CSF mRNA without an IRES or Kozak sequence (GCSF no Kozak; mRNA sequence shown in SEQ ID NO: 7338; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) or a G-CSF sequence having a Kozak sequence (G-CSF Kozak; mRNA sequence shown in SEQ ID NO: 7339; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with fully modified with 5-methylcytidine and 1-methylpseudouridine and tested at a

concentration of 75 ng per well in 24 well plates. 24 hours after transfection, the expression of G-CSF was measured by ELISA, and the results are shown in Table 39.

Table 39. G-CSF expression

Description	Protein Expression (ng/ml)
G-CSF IRES Kozak	2.01
G-CSF IRES	1.64
G-CSF no Kozak	795.53
G-CSF Kozak	606.28

Example 49. MALATl Constructs

[001107] Modified mRNA encoding G-CSF or mCherry with a human or mouse MALATl sequence and their corresponding cDNA sequences are shown below in Table 40. In Table 40, the start codon of each sequence is underlined and the MALATl seuqences are bolded.

Table 40. MALATl Constructs

	Sequence	SEQ ID NO:
G-CSF with Mouse MALAT1 sequence	Optimized G-CSF cDNA sequence containing a T7 polymerase site, kozak sequence, and a Mouse MALAT1 sequence (bold): TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATA TAAGAGCCACC ATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTA TGGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGG ACAGTCCAAGAAGCGACTCCTCTCGGACCTGCCTCAT CGTTGCCGCAGTCATTCCTTTTGAAGTGTCTGGAGCAG GTGCGAAAGATTCAGGGCGATGGAGCCGCACTCCAAG AGAAGCTCTGCGCGACATACAAACTTTGCCATCCCGA GGAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCC TGGGCTCCTCTCTCGTCCTGTCCGTCGCAGGCTTTGCA GTTGGCAGGGTGCCTTTCCCAGCTCCACTCCGGTTTGT TCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGAAT CTCGCCAGAATTGGGCCCGACGCTGGACACGTTGCAG CTCGACGTGGCGGATTTCGCAACAACCATCTGGCAGC AGATGGAGGAACTGGGGATGGCACCCGCGCTGCAGCC CACGCAGGGGGCAATGCCGGCCTTTGCGTCCGCGTTT CAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCACC TTCAATCATTTTTGGAAGTCTCGTACCGGGTGCTGAGA CATCTTGCGCAGCCG TGATAATAG **GATTCGTCAGTAGGGTTGTAAAGGTTTTTCTTTTCC**	7340

	TGAGAAAACAACCTTTTGTTTTCTCAGGTTTTGCTT TTTGGCCTTTCCCTAGCTTTAAAAAAAAAAAAGCAA AAGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCTCTA GA	
	mRNA sequence (transcribed): GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAU AUAAGAGCCACC AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUU AUGGCCCUGCAGUUGCUGCUUUGGCACUCGGCCCUC UGGACAGUCCAAGAAGCGACUCCUCUCGGACCUGCC UCAUCGUUGCCGCAGUCAUUCCUUUUGAAGUGUCUG GAGCAGGUGCGAAAGAUUCAGGGCGAUGGAGCCGCA CUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUGC CAUCCCGAGGAGCUCGUACUGCUCGGGCACAGCUUG GGGAUUCCCUGGGCUCCUCUCUCGUCCUGUCCGUCG CAGGCUUUGCAGUUGGCAGGGUGCCUUUCCCAGCUC CACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCAA GCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACG CUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCA ACAACCAUCUGGCAGCAGAUGGAGGAACUGGGGAUG GCACCCGCGCUGCAGCCCACGCAGGGGGCAAUGCCG GCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGA GUCCUCGUAGCGAGCCACCUUCAAUCAUUUUUGGAA GUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCCG UGAUAAUAG **GAUUCGUCAGUAGGGUUGUAAAGGUUUUUCUUUU CCUGAGAAAACAACCUUUUGUUUUCUCAGGUUUUG CUUUUUGGCCUUUCCCUAGCUUUAAAAAAAAAAAA GC**AAAAGUGGUCUUUG AAUAAAGUCUG AGUGGGC G GC	7341
mCherry with Mouse MALAT1 sequence	Optimized mCherry cDNA sequence containing a T7 polymerase site, kozak sequence, and a Mouse MALAT1 sequence (bold): TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATA TAAGAGCCACC ATGGTATCCAAGGGGGAGGAGGACAACATGGCGATC ATCAAGGAGTTCATGCGATTCAAGGTGCACATGGAAG GTTCGGTCAACGGACACGAATTTGAAATCGAAGGAGA GGGTGAAGGAAGGCCCTATGAAGGGACACAGACCGC GAAACTCAAGGTCACGAAAGGGGGACCACTTCCTTTC GCCTGGGACATTCTTTCGCCCCAGTTTATGTACGGGTC CAAAGCATATGTGAAGCATCCCGCCGATATTCCTGAC TATCTGAAACTCAGCTTTCCCGAGGGATTCAAGTGGG AGCGGGTCATGAACTTTGAGGACGGGGGTGTAGTCAC CGTAACCCAAGACTCAAGCCTCCAAGACGGCGAGTTC ATCTACAAGGTCAAACTGCGGGGGACTAACTTTCCGT CGGATGGGCCGGTGATGCAGAAGAAAACGATGGGAT	7342

	GGGAAGCGTCATCGGAGAGGATGTACCCAGAAGATG GTGCATTGAAGGGGGAGATCAAGCAGAGACTGAAGTT GAAAGATGGGGGACATTATGATGCCGAGGTGAAAAC GACATACAAAGC GAAAAAGCC GGTGC AGCTTC CCGGA GCGTATAATGTGAATATCAAGTTGGATATTACTTCACA CAATGAGGACTACACAATTGTCGAACAGTACGAACGC GCTGAGGGTAGACACTCGACGGGAGGCATGGACGAG TTGTACAAA TGATAATAG **GATTCGTCAGTAGGGTTGTAAAGGTTTTTCTTTTCC TGAGAAAACAACCTTTTGTTTTCTCAGGTTTTGCTT TTTGGCCTTTCCCTAGCTTTAAAAAAAAAAAAGCAA** AAGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCTCTA GA	
	mRNA sequence (transcribed): GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAU AUAAGAGCCACC AUGGUAUCCAAGGGGGAGGAGGACAACAUGGCGAUC AUCAAGGAGUUCAUGCGAUUCAAGGUGCACAUGGAA GGUUC GGUC AAC GGA CA CGAAUUUG AAAUC GAAGG A GAGGGUGAAGGAAGGCCCUAUGAAGGGACACAGACC GCGAAACUCAAGGUCACGAAAGGGGGACCACUUCCU UUCGCCUGGGACAUUCUUUCGCCCCAGUUUAUGUAC GGGUCCAAAGCAUAUGUGAAGCAUCCCGCCGAUAUU CCUGACUAUCUGAAACUCAGCUUUCCCGAGGGAUUC AAGUGGGAGCGGGUCAUGAACUUUGAGGACGGGGG UGUAGUCACCGUAACCCAAGACUCAAGCCUCCAAGA CGGCGAGUUCAUCUACAAGGUCAAACUGCGGGGGAC UAACUUUCCGUCGGAUGGGCCGGUGAUGCAGAAGAA AACGAUGGGAUGGGAAGCGUCAUCGGAGAGGAUGU ACCCAGAAGAUGGUGCAUUGAAGGGGGAGAUCAAGC AGAGACUGAAGUUGAAAGAUGGGGGACAUUAUGAU GCCGAGGUGAAAACGACAUACAAAGCGAAAAAGCCG GUGCAGCUUCCCGGAGCGUAUAAUGUGAAUAUCAAG UUGGAUAUUACUUCACACAAUGAGGACUACACAAUU GUC GAAC AGUAC GAACGC GCUG AGGGUAG ACACUC G ACGGGAGGCAUGGACGAGUUGUACAAA UGAUAAUAG **GAUUCGUCAGUAGGGUUGUAAAGGUUUUUCUUUU CCUGAGAAAACAACCUUUUGUUUUCUCAGGUUUUG CUUUUUGGCCUUUCCCUAGCUUUAAAAAAAAAAAA GC**AAAAGUGGUCUUUG AAUAAAGUCUG AGUGGGC G GC	7343
G-CSF with Human MALAT1	Optimized G-CSF cDNA sequence containing a T7 polymerase site, kozak sequence, and a Human MALAT1 sequence (bold): TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATA	7344

sequence	TAAGAGCCACC ATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTA TGGCCCTGCAGTTGCTGCTTTGGCACTCGGCCCTCTGG ACAGTCCAAGAAGCGACTCCTCTCGGACCTGCCTCAT CGTTGCCGCAGTCATTCCTTTTGAAGTGTCTGGAGCAG GTGCGAAAGATTCAGGGCGATGGAGCCGCACTCCAAG AGAAGCTCTGCGCGACATACAAACTTTGCCATCCCGA GGAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCC TGGGCTCCTCTCTCGTCCTGTCCGTCGCAGGCTTTGCA GTTGGCAGGGTGCCTTTCCCAGCTCCACTCCGGTTTGT TCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGAAT CTCGCCAGAATTGGGCCCGACGCTGGACACGTTGCAG CTCGACGTGGCGGATTTCGCAACAACCATCTGGCAGC AGATGGAGGAACTGGGGATGGCACCCGCGCTGCAGCC CACGCAGGGGGCAATGCCGGCCTTTGCGTCCGCGTTT CAGCGCAGGGCGGGTGGAGTCCTCGTAGCGAGCCACC TTCAATCATTTTTGGAAGTCTCGTACCGGGTGCTGAGA CATCTTGCGCAGCCG TGATAATAG **TGCTCTTCAGTAGGGTCATGAAGGTTTTTCTTTTCC** **TGAGAAAACAACACGTATTGTTTTCTCAGGTTTTGC** **TTTTTGGCCTTTTTCTAGCTTAAAAAAAAAAAAAGC** AAAAGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCTC TAGA	
	mRNA sequence (transcribed): GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAU AUAAGAGCCACC AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUU AUGGCCCUGCAGUUGCUGCUUUGGCACUCGGCCCUC UGGACAGUCCAAGAAGCGACUCCUCUCGGACCUGCC UCAUCGUUGCCGCAGUCAUUCCUUUUGAAGUGUCUG GAGCAGGUGCGAAAGAUUCAGGGCGAUGGAGCCGCA CUCCAAGAGAAGCUCUGCGCGACAUACAAACUUUGC CAUCCCGAGGAGCUCGUACUGCUCGGGCACAGCUUG GGGAUUCCCUGGGCUCCUCUCUCGUCCUGUCCGUCG CAGGCUUUGCAGUUGGCAGGGUGCCUUUCCCAGCUC CACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCAA GCCCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACG CUGGACACGUUGCAGCUCGACGUGGCGGAUUUCGCA ACAACCAUCUGGCAGCAGAUGGAGGAACUGGGGAUG GCACCCGCGCUGCAGCCCACGCAGGGGGCAAUGCCG GCCUUUGCGUCCGCGUUUCAGCGCAGGGCGGGUGGA GUCCUCGUAGCGAGCCACCUUCAAUCAUUUUUGGAA GUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCCG UGAUAAUAG **UGCUCUUCAGUAGGGUCAUGAAGGUUUUUCUUUUC**	7345

	CUGAGAAAACAACACGUAUUGUUUUCUCAGGUUUU GCUUUUUGGCCUUUUUCUAGCUUAAAAAAAAAAAAA AGCAAAAGUGGUCUUUGAAUAAAGUCUGAGUGGGC GGC	
mCherry with Human MALAT1 sequence	Optimized mCherry cDNA sequence containing a T7 polymerase site, kozak sequence, and a Human MALAT1 sequence (bold): TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATA TAAGAGCCACC ATGGTATCCAAGGGGGAGGAGGACAACATGGCGATC ATCAAGGAGTTCATGCGATTCAAGGTGCACATGGAAG GTTCGGTCAACGGACACGAATTTGAAATCGAAGGAGA GGGTGAAGGAAGGCCCTATGAAGGGACACAGACCGC GAAACTCAAGGTCACGAAAGGGGGACCACTTCCTTTC GCCTGGGACATTCTTTCGCCCCAGTTTATGTACGGGTC CAAAGCATATGTGAAGCATCCCGCCGATATTCCTGAC TATCTGAAACTCAGCTTTCCCGAGGGATTCAAGTGGG AGCGGGTCATGAACTTTGAGGACGGGGGTGTAGTCAC CGTAACCCAAGACTCAAGCCTCCAAGACGGCGAGTTC ATCTACAAGGTCAAACTGCGGGGGACTAACTTTCCGT CGGATGGGCCGGTGATGCAGAAGAAAACGATGGGAT GGGAAGCGTCATCGGAGAGGATGTACCCAGAAGATG GTGCATTGAAGGGGGAGATCAAGCAGAGACTGAAGTT GAAAGATGGGGGACATTATGATGCCGAGGTGAAAAC GACATACAAAGCGAAAAAGCCGGTGCAGCTTCCCGGA GCGTATAATGTGAATATCAAGTTGGATATTACTTCACA CAATGAGGACTACACAATTGTCGAACAGTACGAACGC GCTGAGGGTAGACACTCGACGGGAGGCATGGACGAG TTGTACAAA TGATAATAG **TGCTCTTCAGTAGGGTCATGAAGGTTTTTCTTTTCC TGAGAAAACAACACGTATTGTTTTCTCAGGTTTTGC TTTTTGGCCTTTTTCTAGCTTAAAAAAAAAAAAAGC** AAAAGTGGTCTTTGAATAAAGTCTGAGTGGGCGGCTC TAGA	7346
	mRNA sequence (transcribed): GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAU AUAAGAGCCACC AUGGUAUCCAAGGGGGAGGAGGACAACAUGGCGAUC AUCAAGGAGUUCAUGCGAUUCAAGGUGCACAUGGAA GGUUCGGUCAACGGACACGAAUUUGAAAUCGAAGGA GAGGGUGAAGGAAGGCCCUAUGAAGGGACACAGACC GCGAAACUCAAGGUCACGAAAGGGGGACCACUUCCU UUCGCCUGGGACAUUCUUUCGCCCCAGUUUAUGUAC GGGUCCAAAGCAUAUGUGAAGCAUCCCGCCGAUAUU CCUGACUAUCUGAAACUCAGCUUUCCCGAGGGAUUC AAGUGGGAGCGGGUCAUGAACUUUGAGGACGGGGG	7347

	UGUAGUCACCGUAACCCAAGACUCAAGCCUCCAAGA CGGCGAGUUCAUCUACAAGGUCAAACUGCGGGGGAC UAACUUUCCGUCGGAUGGGCCGGUGAUGCAGAAGAA AACGAUGGGAUGGGAAGCGUCAUCGGAGAGGAUGU ACCCAGAAGAUGGUGCAUUGAAGGGGGAGAUCAAGC AGAGACUGAAGUUGAAAGAUGGGGGACAUUAUGAU GCCGAGGUGAAAACGACAUACAAAGCGAAAAAGCCG GUGCAGCUUCCCGGAGCGUAUAAUGUGAAUAUCAAG UUGGAUAUUACUUCACACAAUGAGGACUACACAAUU GUCGAACAGUACGAACGCGCUGAGGGUAGACACUCG ACGGGAGGCAUGGACGAGUUGUACAAA UGAUAAUAG **UGCUCUUCAGUAGGGUCAUGAAGGUUUUUCUUUUC CUGAGAAAACAACACGUAUUGUUUUCUCAGGUUUU GCUUUUUGGCCUUUUUCUAGCUUAAAAAAAAAAAA** AGCAAAAGUGGUCUUUGAAUAAAGUCUGAGUGGGC GGC	

[001108] These modified mRNA sequences can include at least one chemical modification described herein. The G-CSF or mCherry modified mRNA sequence can be formulated, using methods described herein and/or known in the art, prior to transfection and/or administration.

[001109] The modified mRNA sequence encoding G-CSF or mCherry can be transfected *in vitro* to various cell types such as HEK293, HeLa, PBMC and BJ fibroblast and those described in Table 25 of co-pending US Provisional Application No. 61/839,903, filed June 27th, 2013, the contents of which are herein incorporated by reference in its entirety, using methods disclosed herein and/or are known in the art. The cells are then analyzed using methods disclosed herein and/or are known in the art to determine the concentration of G-CSF or mCherry and/or the cell viability.

Example 50. Oncology-related Targets

[001110] Septin 4 may be an oncology-related polypeptide of interest. Shown in Table 41, in addition to the name and description of the gene encoding the oncology-related polypeptide of interest, are the ENSEMBL Transcript ID (ENST), the ENSEMBL Protein ID (ENSP), each present where applicable, and when available the optimized sequence ID (OPT. SEQ ID).

Table 41. Oncology-Related Targets

Target	Target Description	ENST ID	Trans. SEQ ID NO	ENSP ID	Prot. SEQ ID NO	OPT. SEQ ID NO
SEPT4	septin 4	317256	7348	321071	7355	7363, 7368, 7375, 7382, 7389, 7396
SEPT4	septin 4	317268	7349	321674	7356	7364, 7369, 7376, 7383, 7390, 7397, 7403-7489
SEPT4	septin 4	393086	7350	376801	7357	7370, 7377, 7384, 7391, 7398
SEPT4	septin 4	412945	7351	414779	7358	7365, 7371, 7378, 7385, 7392, 7399
SEPT4	septin 4	426861	7352	402348	7359	7366, 7372, 7379, 7386,7393, 7400
SEPT4	septin 4	457347	7353	402000	7360	7367, 7373, 7380, 7387, 7394, 7401
SEPT4	septin 4	583114	7354	463768	7361	7374, 7381, 7388, 7395, 7402
SEPT4	septin 4					7362

Example 51. Confirmation and of Peptide Identity from Chemically Modified mRNA

[001111] Cell lysates containing protein produced from: (a) apoptosis-inducing factor 1, mitochondrial, short isoform (AIFsh; gene name AIFM1) modified mRNA (mRNA sequence shown in SEQ ID NO. 6617 (Table 42); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl); (b) copper metabolism (Murrl) domain containing 1 (COMMD1) modified mRNA (mRNA sequence shown in SEQ ID NO. 7491 (Table 42); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl); (c) septin 4 (SEPT4) modified mRNA (mRNA sequence shown in SEQ ID NO. 7362 (Table 42); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl); and (d) diablo, IAP-binding mitochondrial protein (DIABLO) modified mRNA (mRNA sequence shown in SEQ ID NO. 7494 (Table 42); polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl); all fully modified with 5-methylcytidine and pseudouridine (5mC and pU), fully modified with 5-methylcytidine and 1-methylpseudouridine (5mC and ImpU), modified where 25% of uridine modified with 2-thiouridine and 25% of cytidine modified with 5-methylcytidine (s2U and 5mC), fully modified with pseudouridine (pU), or fully modified with 1-methylpseudouridine (ImpU) were evaluated using the LC-MS/MS with quantitative LC-MRM as described in Example 31.

Table 42. Target Sequences

Description	Sequence	SEQ ID NO:
AIFsh	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAU AAGAGCCACCAUGGAAAAAGUCAGACGAGAGGGGGUU AAGGUGAUGCCCAAUGCUAUUGUGCAAUCCGUUGGAG UCAGCAGUGGCAAGUUACUUAUCAAGCUGAAAGACGG CAGGAAGGUAGAAACUGACCACAUAGUGGCAGCUGUG GGCCUGGAGCCCAAUGUUGAGUUGGCCAAGACUGGUG GCCUGGAAAUAGACUCAGAUUUUGGUGGCUUCCGGGU AAAUGCAGAGCUACAAGCACGCUCUAACAUCUGGGUG GCAGGAGAUGCUGCAUGCUUCUACGAUAUAAAGUUGG GAAGGAGGCGGGUAGAGCACCAUGAUCACGCUGUUGU GAGUGGAAGAUUGGCUGGAGAAAAUAUGACUGGAGCU GCUAAGCCGUACUGGCAUCAGUCAAUGUUCUGGAGUG AUUUGGGCCCCGAUGUUGGCUAUGAAGCUAUUGGUCU UGUGGACAGUAGUUUGCCCACAGUUGGUGUUUUUGCA AAAGCAACUGCACAAGACAACCCCAAAUCUGCCACAGA GCAGUCAGGAACUGGUAUCCGAUCAGAGAGUGAGACA GAGUCCGAGGCCUCAGAAAUUACUAUUCCUCCCAGCAC CCCGGCAGUUCCACAGGCUCCCGUCCAGGGGGAGGACU ACGGCAAAGGUGUCAUCUUCUACCUCAGGGACAAAGU GGUCGUGGGGAUUGUGCUAUGGAACAUCUUUAACCGA AUGCCAAUAGCAAGGAAGAUCAUUAAGGACGGUGAGC AGCAUGAAGAUCUCAAUGAAGUAGCCAAACUAUUCAA CAUUCAUGAAGACUGAUAAUAGGCUGGAGCCUCGGUG GCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCAGCCCCU CCUCCCCUUCCUGCACCCGUACCCCCGUGGUCUUUGAA UAAAGUCUGAGUGGGCGGC	6617
	MEKVRREGVKVMPNAIVQSVGVSSGKLLIKLKDGRK VETDHIVAAVGLEPNVELAKTGGLEIDSDFGGFRVNA ELQARSNIWVAGDAACFYDIKLGRRRVEHHDHAVVS GRLAGENMTGAAKPYWHQSMFWSDLGPDVGYEAIG LVDSSLPTVGVFAKATAQDNPKSATEQSGTGIRSESET ESEASEITIPPSTPAVPQAPVQGEDYGKGVIFYLRDKV VVGIVLWNIFNRMPIARKIIKDGEQHEDLNEVAKLFNI HED	7490
COMMD1	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAA UAUAAGAGCCACCAUGGCGGCGGGCGAGCUUGAG GGUGGCAAACCCCUGAGCGGGCUGCUGAAUGCGC UGGCCCAGGACACUUUCCACGGGUACCCCGGCAUC ACAGAGGAGCUGCUACGGAGCCAGCUAUAUCCAG AGGUGCCACCCGAGGAGUUCCGCCCCUUUCUGGCA AAGAUGAGGGGGAUUCUUAAGUCUAUUGCGUCUG CAGACAUGGAUUUCAACCAGCUGGAGGCAUUCUU GACUGCUCAAACCAAAAAGCAAGGUGGGAUCACA UCUGACCAAGCUGCUGUCAUUUCCAAAUUCUGGA AGAGCCACAAGACAAAAAUCCGUGAGAGCCUCAU	7491

	GAACCAGAGCCGCUGGAAUAGCGGGCUUCGGGGC CUGAGCUGGAGAGUUGAUGGCAAGUCUCAGUCAA GGCACUCAGCUCAAAUACACACCUGUUGCCAU UAUAGAGCUGGAAUUAGGCAAAUAUGGACAGGAA UCUGAAUUUCUGUGUUUGGAAUUUGAUGAGGUCA AAGUCAACCAAAUUCUGAAGACGCUGUCAGAGGU AGAAGAAAGUAUCAGCACACUGAUCAGCCAGCCU AACUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGC UUCUUGCCCCUUGGGCCUCCCCCCAGCCCCUCCUC CCCUUCCUGCACCCGUACCCCCGUGGUCUUUGAAU AAAGUCUGAGUGGGCGGC	
	MAAGELEGGKPLSGLLNALAQDTFHGYPGITEELLRS QLYPEVPPEEFRPFLAKMRGILKSIASADMDFNQLEAF LTAQTKKQGGITSDQAAVISKFWKSHKTKIRESLMNQ SRWNSGLRGLSWRVDGKSQSRHSAQIHTPVAIIELELG KYGQESEFLCLEFDEVKVNQILKTLSEVEESISTLISQP N	7492
SEPT4	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAA UAUAAGAGCCACCAUGGACCGUUCACUGGGAUGG CAAGGGAAUUCUGUCCCUGAGGACAGGACUGAAG CUGGGAUCAAGCGUUUCCUGGAGGACACCACGGA UGAUGGAGAACUGAGCAAGUUCGUGAAGGAUUUC UCAGGAAAUGCGAGCUGCCACCCACCAGAGGCUA AGACCUGGGCAUCCAGGCCCCAAGUCCCGGAGCCA AGGCCCCAGGCCCCGGACCUCUAUGAUGAUGACCU GGAGUUCAGACCCCCCUCGCGGCCCCAGUCCUCUG ACAACCAGCAGUACUUCUGUGCCCCAGCCCCUCUC AGCCCAUCUGCCAGGCCCCGCAGCCCAUGGGGCAA GCUUGAUCCCUAUGAUUCCUCUGAGGAUGACAAG GAGUAUGUGGGCUUUGCAACCCUCCCCAACCAAG UCCACCGAAAGUCCGUGAAGAAAGGCUUUGACUU UACCCUCAUGGUGGCAGGAGAGUCUGGCCUGGGC AAAUCCACACUUGUCAAUAGCCUCUUCCUCACUG AUCUGUACCGGGACCGGAAACUUCUUGGUGCUGA AGAGAGGAUCAUGCAAACUGUGGAGAUCACUAAG CAUGCAGUGGACAUAGAAGAGAAGGGUGUGAGGC UGCGGCUCACCAUUGUGGACACACCAGGUUUUGG GGAUGCAGUCAACAACACAGAGUGCUGGAAGCCU GUGGCAGAAUACAUUGAUCAGCAGUUUGAGCAGU AUUUCCGAGACGAGAGUGGCCUGAACCGAAAGAA CAUCCAAGACAACAGGGUGCACUGCUGCCUGUAC UUCAUCUCACCCUUCGGCCAUGGGCUCCGGCCAUU GGAUGUUGAAUUCAUGAAGGCCCUGCAUCAGCGG GUCAACAUCGUGCCUAUCCUGGCUAAGGCAGACA CACUGACACCUCCCGAAGUGGACCACAAGAAACGC AAAAUCCGGGAGGAGAUUGAGCAUUUUGGAAUCA	7362

	AGAUCUAUCAAUUCCCAGACUGUGACUCUGAUGA GGAUGAGGACUUCAAAUUGCAGGACCAAGCCCUA AAGGAAAGCAUCCCAUUUGCAGUAAUUGGCAGCA ACACUGUAGUAGAGGCCAGAGGGCGGCGAGUUCG GGGUCGACUCUACCCCUGGGGCAUCGUGGAAGUG GAAAACCCAGGGCACUGCGACUUUGUGAAGCUGA GGACAAUGCUGGUACGUACCCACAUGCAGGACCU GAAGGAUGUGACACGGGAGACACAUUAUGAGAAC UACCGGGCACAGUGCAUCCAGAGCAUGACCCGCCU GGUGGUGAAGGAACGGAAUCGCAACAAACUGACU CGGGAAAGUGGUACCGACUUCCCCAUCCCUGCUG UCCCACCAGGGACAGAUCCAGAAACUGAGAAGCU UAUCCGAGAGAAAGAUGAGGAGCUGCGGCGGAUG CAGGAGAUGCUACACAAAAUACAAAAACAGAUGA AGGAGAACUAUUGAUAAUAGGCUGGAGCCUCGGU GGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCAGC CCCUCCUCCCCUUCCUGCACCCGUACCCCCGUGGU CUUUGAAUAAAGUCUGAGUGGGCGGC	
	MDRSLGWQGNSVPEDRTEAGIKRFLEDTTDDGELSKF VKDFSGNASCHPPEAKTWASRPQVPEPRPQAPDLYDD DLEFRPPSRPQSSDNQQYFCAPAPLSPSARPRSPWGKL DPYDSSEDDKEYVGFATLPNQVHRKSVKKGFDFTLM VAGESGLGKSTLVNSLFLTDLYRDRKLLGAEERIMQT VEITKHAVDIEEKGVRLRLTIVDTPGFGDAVNNTECW KPVAEYIDQQFEQYFRDESGLNRKNIQDNRVHCCLYF ISPFGHGLRPLDVEFMKALHQRVNIVPILAKADTLTPP EVDHKKRKIREEIEHFGIKIYQFPDCDSDEDEDFKLQD QALKESIPFAVIGSNTVVEARGRRVRGRLYPWGIVEV ENPGHCDFVKLRTMLVRTHMQDLKDVTRETHYENY RAQCIQSMTRLVVKERNRNKLTRESGTDFPIPAVPPGT DPETEKLIREKDEELRRMQEMLHKIQKQMKENY	7493
Diablo, IAP-binding mitochondrial protein (DIABLO)	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAA UAUAAGAGCCACCAUGGCGGCUCUGAAGAGUUGG CUGUCGCGCAGCGUAACUUCAUUCUUCAGGUACA GACAGUGUUUGUGUGUUCCUGUUGUGGCUAACUU UAAGAAGCGGUGUUUCUCAGAAUUGAUAAGACCA UGGCACAAAACUGUGACGAUUGGCUUUGGAGUAA CCCUGUGUGCGGUUCCUAUUGCACAGAAAUCAGA GCCUCAUUCCCUUAGUAGUGAAGCAUUGAUGAGG AGAGCAGUGUCUUUGGUAACAGAUAGCACCUCUA CCUUUCUCUCUCAGACCACAUAUGCGUUGAUUGA AGCUAUUACUGAAUAUACUAAGGCUGUUUAUACC UUAACUUCUCUUUACCGACAAUAUACAAGUUUAC UUGGGAAAAUGAAUUCAGAGGAGGAAGAUGAAGU GUGGCAGGUGAUCAUAGGAGCCAGAGCUGAGAUG ACUUCAAAACACCAAGAGUACUUGAAGCUGGAAA	7494

	CCACUUGGAUGACUGCAGUUGGUCUUUCAGAGAU GGCAGCAGAAGCUGCAUAUCAAACUGGCGCAGAU CAGGCCUCUAUAACCGCCAGGAAUCACAUUCAGC UGGUGAAACUGCAGGUGGAAGAGGUGCACCAGCU CUCCCGGAAAGCAGAAACCAAGCUGGCAGAAGCA CAGAUAGAAGAGCUCCGUCAGAAAACACAGGAGG AAGGGGAGGAGCGGGCUGAGUCGGAGCAGGAGGC CUACCUGCGUGAGGAUUGAUAAUAGGCUGGAGCC UCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCC CCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCC GUGGUCUUUG AAUAAAGUCUG AGUGGGC GGC	
	MAALKSWLSRSVTSFFRYRQCLCVPVVANFKKRCFSE LIRPWHKTVTIGFGVTLCAVPIAQKSEPHSLSSEALMR RAVSLVTDSTSTFLSQTTYALIEAITEYTKAVYTLTSLY RQYTSLLGKMNSEEEDEVWQVIIGARAEMTSKHQEY LKLETTWMTAVGLSEMAAEAAYQTGADQASITARNH IQLVKLQVEEVHQLSRKAETKLAEAQIEELRQKTQEE GEERAESEQEAYLRED	1986

[001112] Peptide fragments identified for the evaluated proteins are shown in Table 43.

Table 43. Protein and Peptide Fragment Sequences

	Peptide Fragment SEQ ID NO	5mC and pU	5mC and 1mpU	s2U and 5mC	pU	1mpU
AIFM1						
DGEQHEDLNEVAK	7495	-	-	-	-	YES
TGGLEIDSDFGGFR	7496	YES	-	-	YES	YES
COMMD1						
ESLMNQSR	7497	YES	YES	YES	YES	YES
HSAQIHTPVAIIELELGK	7498	-	-	YES	YES	YES
WNSGLR	7499	-	YES	YES	YES	YES
SEPT4						
ESGTDFPIPAVPPGTDPETEK	7500	YES	YES	YES	YES	YES
FLEDTTDDGELSK	7501	YES	YES	YES	YES	YES
HAVDIEEK	7502	YES	YES	YES	YES	YES
DIABLO						
AVYTLTSLYR	7503	YES	YES	YES	YES	YES
LAEAQIEELR	7504	YES	YES	YES	YES	YES
NHIQLVK	7505	YES	YES	YES	YES	YES

Example 52. Detection of C.A. Caspase 3 and C.A Caspase 6

[001113] Human lung cancer A549 cells were plated in 6-wells, and transfected with Lipofectamine 2000 (Life Technologies) and 5 μg of constitutively active (C.A.) caspase 3 mRNA (mRNA sequence shown in SEQ ID NO: 6619 (Table 44); polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl) or constitutively active (C.A.) caspase 6 mRNA (mRNA sequence shown in SEQ ID NO: 7506 (Table 44); polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl) fully modified with 5-methylcytidine and 1-methylpseudouridine (5mC and ImpU) or fully modified with 1-methylpseudouridine (ImpU). Cells were harvested 7-10 hours post-transfection and lysed in RIPA buffer containing a protease inhibitor cocktail (Roche, Indianapolis, IN). 20 μg of cell lysate per lane was run for Western blotting to detect endogenous and introduced caspase 3; endogenous and introduced caspase 6; the caspase 3 downstream-substrate PARP; and the caspase 6 downstream-substrate lamin A/C. Compared to control lysate, higher levels of cleaved caspase 3 and cleaved caspase 6 were detected in C.A. caspase 3 and C.A. caspase 6 modified mRNA transfected cells, respectively. As shown in Figure 7, cleavage of the downstream substrates PARP and lamin A/C were detected in cells treated with C.A. caspase 3 modified mRNA (Figure 7A) and C.A. caspase 6 modified mRNAs (Figure 7B). The 5 lanes of the Westerns shown in Figure 7A and 7B contain lysate from the following: 1) untransfected HeLa cells, 2) untransfected A549, 3) A549 lipofectamine alone control, 4) A549 transfected with 5mC, ImpU modified mRNA, and 5) A549 transfected with ImpU modified mRNA.

Table 44. C.A. Caspase Sequences

Description	Sequence	SEQ ID NO:
C.A. caspase 3	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUA UAAGAGCCACCAUGAUUGAGACAGACAGUGGUGUUGA UGAUGACAUGGCGUGUCAUAAAAUACCAGUGGAGGCC GACUUCUUGUAUGCAUACUCCACAGCACCUGGUUAUU AUUCUUGGCGAAAUUCAAAGGAUGGCUCCUGGUUCAU CCAGUCGCUUUGUGCCAUGCUGAAACAGUAUGCCGAC AAGCUUGAAUUUAUGCACAUUCUUACCCGGGUUAACC GAAAGGUGGCAACAGAAUUUGAGUCCUUUUCCUUUGA CGCUACUUUUCAUGCAAAGAAACAGAUUCCAUGUAUU	6619

	GUUUCCAUGCUCACAAAAGAACUCUAUUUUUAUCACG AUGAAGUUGAUGGGGGAUCCCCCAUGGAGAACACUGA AAACUCAGUGGAUUCAAAAUCCAUUAAAAAUUUGGA ACCAAAGAUCAUACAUGGAAGCGAAUCAAUGGACUCU GGAAUAUCCCUGGACAACAGUUAUAAAAUGGAUUAUC CUGAGAUGGGUUUAUGUAUAAUAAUUAAUAAUAAGA AUUUUCAUAAGAGCACUGGAAUGACAUCUCGGUCUGG UACAGAUGUCGAUGCAGCAAACCUCAGGGAAACAUUC AGAAACUUGAAAUAUGAAGUCAGGAAUAAAAAUGAU CUUACACGUGAAGAAAUUGUGGAAUUGAUGCGUGAU GUUUCUAAAGAAGAUCACAGCAAAAGGAGCAGUUUU GUUUGUGUGCUUCUGAGCCAUGGUGAAGAAGGAAUA AUUUUUGGAACAAAUGGACCUGUUGACCUGAAAAAA AUAACAAACUUUUUCAGAGGGGAUCGUUGUAGAAGU CUAACUGGAAAACCCAAACUUUUCAUUAUUCAGGCCU GCCGUGGUACAGAACUGGACUGUGGCAUUGAGACAGA CUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUU GCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCU GCACCCGUACCCCCGUGGUCUUUGAAUAAAGUCUGAG UGGGCGGC	
	MIETDSGVDDDMACHKIPVEADFLYAYSTAPGYYSW RNSKDGSWFIQSLCAMLKQYADKLEFMHILTRVNRK VATEFESFSFDATFHAKKQIPCIVSMLTKELYFYHDE VDGGSPMENTENSVDSKSIKNLEPKIIHGSESMDSGIS LDNSYKMDYPEMGLCIIINNKNFHKSTGMTSRSGTD VDAANLRETFRNLKYEVRNKNDLTREEIVELMRDVS KEDHSKRSSFVCVLLSHGEEGIIFGTNGPVDLKKITNF FRGDRCRSLTGKPKLFIIQACRGTELDCGIETD	2484
C.A. caspase 6	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAA AUAUAAGAGCCACCAUGGUAGAAAUAGAUGCAGC CUCCGUUUACACGCUGCCUGCUGGAGCUGACUUC CUCAUGUGUUACUCUGUUGCAGAAGGAUAUUAU UCUCACCGGGAAACUGUGAACGGCUCAUGGUACA UUCAAGAUUUGUGUGAGAUGUUGGGAAAAUAUG GCUCCUCCUUAGAGUUCACAGAACUCCUCACACU GGUGAACAGGAAAGUUUCUCAGCGCCGAGUGGAC UUUUGCAAAGACCCAAGUGCAAUUGGAAAGAAGC AGGUUCCCUGUUUUGCCUCAAUGCUAACUAAAAA GCUGCAUUUCUUUCCAAAAUCUAAUCUCGAGCAC CACCACCACCACCACGUUGAAAUUGAUGGGGGAU CCCCCAUGAGCUCGGCCUCGGGGCUCCGCAGGGG GCACCCGGCAGGUGGGGAAGAAAACAUGACAGAA ACAGAUGC CUUCU AUAAAAG AGAAAUGUUUG AU CCGGCAGAAAAGUACAAAAUGGACCACAGGAGGA GAGGAAUUGCUUUAAUCUUCAAUCAUGAGAGGU UCUUUUGGCACUUAACACUGCCAGAAAGGCGGGG CACCUGCGCAGAUAGAGACAAUCUUACCCGCAGG UUUUCAGAUCUAGGAUUUGAAGUGAAAUGCUUU	7506

	AAUGAUCUUAAAGCAGAAGAACUACUGCUCAAAA UUCAUGAGGUGUCAACUGUUAGCCACGCAGAUGC CGAUUGCUUUGUGUGUGUCUUCCUGAGCCAUGGC GAAGGCAAUCACAUUUAUGCAUAUGAUGCUAAA AUCGAAAUUCAGACAUUAACUGGCUUGUUCAAAG GAGACAAGUGUC ACAGC CUGGUUGG AAAAC CCAA GAUAUUUAUCAUCCAGGCAUGUCGGGGAAACCAG CACGAUGUGCCAGUCAUUCCUUUGGAUGUAGUAG AUUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCU UCUUGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCC CCUUCCUGCACCCGUACCCCCGUGGUCUUUGAAU AAAGUCUGAGUGGGCGGC	
	MVEIDAASVYTLPAGADFLMCYSVAEGYYSHRETVN GSWYIQDLCEMLGKYGSSLEFTELLTLVNRKVSQRR VDFCKDPSAIGKKQVPCFASMLTKKLHFFPKSNLEHH HHHHVEIDGGSPMSSASGLRRGHPAGGEENMTETDA FYKREMFDPAEKYKMDHRRPvGIALIFNHERFFWHLT LPERRGTCADRDNLTRRFSDLGFEVKCFNDLKAEELL LKIHEVSTVSHADADCFVCVFLSHGEGNHIYAYDAKI EIQTLTGLFKGDKCHSLVGKPKIFIIQACRGNQHDVPV IPLDVVD	2486

Example 53. Expression of Modified C.A. Caspase 3 and C.A. Caspase 6 mRNA

[001114] The activity of cultured human lung adenocarcinoma A549 cells was evaluated through the measurement of formazan converted by mitochondrial dehydrogenases from WST-1 substrate (Roche, Indianapolis, IN). 7500 cells per 96-well were treated with a single dose of varying amounts of Lipofectamine 2000-lipoplexed constitutively active (C.A.) caspase 3 mRNA (mRNA sequence shown in SEQ ID NO: 6619 (Table 44); polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl) or constitutively active (C.A.) caspase 6 mRNA (mRNA sequence shown in SEQ ID NO: 7506 (Table 44); polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl) fully modified with 5-methylcytidine and 1-methylpseudouridine (5mC and ImpU) or fully modified with 1-methylpseudouridine (ImpU) or a control proteins (eGFP (mRNA sequence shown in SEQ ID NO: 7507; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl; fully modified with 5-methylcytidine and 1-methylpseudouridine (5mC and ImpU) or fully modified with 1-methylpseudouridine (ImpU)) and luciferase (mRNA sequence shown in SEQ ID NO: 7508; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl;

fully modified with 5-methylcytidine and 1-methylpseudouridine (5mC and ImpU) or fully modified with 1-methylpseudouridine (ImpU))). Cellular activity was measured in cultured cells 1 day after mRNA treatment according to the WST-1 manufacturer's protocol, and is plotted as a 450 nm absorbance reading of the converted formazan that had been corrected for background signal. As shown in Table 45, increasing amounts of transfected C.A. caspase mRNA (0 ng, 2 ng, 10 ng, 50 ng and 250 ng) markedly inhibited the optical density (OD) signal (as a readout of cellular activity) compared to controls. Similar results were obtained in human lung adenocarcinoma H441 cells and human cervical cancer HeLa cells.

Table 45. Cellular Activity

Description	Amount of mRNA (ng)	WST-1 OD mean (450-690 nm)
C.A. caspase 3 (1mpU and 5mC)	0	2.46
	2	1.93
	10	1.05
	50	0.31
	250	0.04
C.A. caspase 6 (1mpU and 5mC)	0	2.49
	2	2.41
	10	1.64
	50	0.75
	250	0.30
eGFP (1mpU and 5mC)	0	2.37
	2	2.36
	10	2.19
	50	1.84
	250	1.84
Luciferase (1mpU and 5mC)	0	2.26
	2	1.93
	10	2.00
	50	1.94
	250	1.87
C.A. caspase 3 (1mpU)	0	2.62
	2	2.35
	10	1.80
	50	0.91
	250	0.17
C.A. caspase 6 (1mpU)	0	2.17
	2	2.34
	10	2.01

	50	1.29
	250	0.42
eGFP (ImpU)	0	2.56
	2	2.67
	10	2.86
	50	2.72
	250	2.38
Luciferase (ImpU)	0	2.12
	2	2.56
	10	2.68
	50	2.64
	250	2.21

Example 54. MYC Inhibitors modified mRNA

[001115] Human hepatocellular carcinoma Hep3B cells were plated in a 6-well plate at a seeding density of 3xl0 ^{6}cells/well and Lipofectamine 2000-transfected with mRNAs fully modified with 5-methylcytidine and 1-methylpseudouridine (5mC and ImpU) or fully modified with 1-methylpseudouridine (ImpU) designed to encode the following: fluorescent protein mCherry (mRNA sequence shown in SEQ ID NO: 6602; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl), non-translatable Factor IX (mRNA sequence shown in SEQ ID NO: 7509; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl), full length wildtype C-MYC (mRNA sequence shown in SEQ ID NO: 7510; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl), MYC inhibitor A (mRNA sequence shown in SEQ ID NO: 751 1 (Table 46); polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl), MYC inhibitor B (mRNA sequence shown in SEQ ID NO: 7513 (Table 46); polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl), MYC inhibitor C (mRNA sequence shown in SEQ ID NO: 7418 (Table 46); polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl) and MYC inhibitor D (mRNA sequence shown in SEQ ID NO: 7515 (Table 46); polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl). Cells were collected 8 hours post-transfection and lysates were made using RIPA lysis buffer including a protease inhibitor cocktail (Roche, Indianapolis,IN). Equal amounts of lysate determined by BCA assay were resolved by SDS-PAGE through 4-12% BIS-TRIS gels, transferred to nitrocellulose blots and probed with appropriate primary and secondary

antibodies. Western blot analyses revealed positive expression of the 4 modified mRNA MYC inhibitors, as well as full length C-MYC, in Hep3B cells.

Table 46. MYC Inhibitor Sequences

Description	Sequence	SEQ ID NO:
MYC inhibitor A	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAA AUAUAAGAGCCACCAUGACCGAAGAAAACGUCAA GAGAAGAACCCAUAAUGUCCUCGAGCGCCAGCGG CGCAAUGAGCUCAAGCGCAGCUUCUUUGCACUCA GGGACCAAAUUCCAGAGUUGGAGAACAACGAAAA GGCCCCGAAGGUGGUGAUCCUUAAGAAGGCGACU GCCUACAUCCUGUCGGUGCAGGCUGAGACUCAAA AGCUGAUCUCCGAAAUCGAUCUGCUCCGGAAACA GAACGAACAACUGAAACACAAACUGGAACAGCUG CGGAAUUCAUGCGCGUGAUAAUAGGCUGGAGCCU CGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCC CCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCC GUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC	7511
	MTEENVKRRTHNVLERQRRNELKRSFFALRDQIPELE NNEKAPKVVILKKATAYILSVQAETQKLISEIDLLRKQ NEQLKHKLEQLRNSCA	7512
MYC inhibitor B	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAA AUAUAAGAGCCACCAUGACCGAAGAAAACGUCAA GAGAAGAACCCAUAAUGUCCUCGAGCGCCAGCGG CGCAAUGAGCUCAAGCGCAGCUUCUUUGCACUCA GGGACCAAAUUCCAGAGUUGGAGAACAACGAAAA GGCCCCGAAGGUGGUGAUCCUUAAGAAGGCGACU GCCUACAUCCUGUCGGUGCAGGCUGAGAAUCAAA AGCUGAUCUCCGAAAUCGAUCUGCUCCGGAAACA GAACGAACAACUGAAACACAAACUGGAACAGCUG CGGAAUUCAUGCGCGUGAUAAUAGGCUGGAGCCU CGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCC CCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCC GUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC	7513
	MTEENVKRRTHNVLERQRRNELKRSFFALRDQIPELE NNEKAPKVVILKKATAYILSVQAENQKLISEIDLLRK QNEQLKHKLEQLRNSCA	7514
MYC inhibitor C	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAA AUAUAAGAGCCACCAUGAGCGCCGCUGAUAAGCG GGCUCACCACAAUGCGUUGGAGAGGAAGAGGCGC GACCACAUCAAAGACUCGUUCCAUUCACUCCGGG ACUCCGUGCCGUCGCUGCAAGGAGAAAAAGCCUC CCGGGCACAGAUCCUCGACAAGGCGACUGAGUAC AUUCAGUACAUGCGCCGCAAGAACCACACCCAUC AGCAAGAUAUCGACGAUCUUAAGAGACAGAACGC	7515

	GCUGCUGG AAC AAC AGGUC CGC GCACUGG AAAAG GCCAGAAGCUCAGCCUGAUAAUAGGCUGGAGCCU CGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCC CCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCC GUGGUCUUUG AAUAAAGUCUG AGUGGGC GGC	
	MSAADKRAHHNALERKRRDHIKDSFHSLRDSVPSLQ GEKASRAQILDKATEYIQYMRRKNHTHQQDIDDLKR QN ALLEQ QVRALEKARS SA	7516
MYC inhibitor D	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUA UAAGAGCCACCAUGACCGAAGAAAACGUCAAGAGAAG AACCCAUAAUGUCCUCGAGCGCCAGCGGCGCAAUGAG CUCAAGCGCAGCUUCUUUGCACUCAGGGACCAAAUUC CAGAGUUGGAGAACAACGAAAAGGCCCCGAAGGUGGU GAUCCUUAAGAAGGCGACUGCCUACAUCCUGUCGGUG CAGGCUGAGACUCAAAAGCUGAUCUCCGAAAUCGAUC UGCUCCGGAAACAGAACGAACAACUGAAACACAAACU GGAACAGCUGCGGAAUUCAUGCUGAUAAUAGGCUGGA GCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCC CCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCGU GGUCUUUGAAUAAAGUCUGAGUGGGCGGC	6621
	MTEENVKRRTHNVLERQRRNELKRSFFALRDQIPELE NNEKAPKVVILKKATAYILSVQAETQKLISEIDLLRKQ NEQLKHKLEQLRNSC	7517

Example 55. Expression of MYC inhibitors Modified mRNA

[001116] Hep3B cells were plated in a 96-well plate at a seeding density of 2500 cells/well and Lipofectamine 2000-transfected with 0, 0.2 nM, 0.7 nM, 2 nM or 6 nM of modified mRNAs fully modified with 1-methylpseudouridine (ImpU) designed to encode the following: mCherry (mRNA sequence shown in SEQ ID NO: 6602; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl), non-translatable Factor IX (mRNA sequence shown in SEQ ID NO: 7509; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl), full length wildtype C-MYC (mRNA sequence shown in SEQ ID NO: 7510; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl), MYC inhibitor A (mRNA sequence shown in SEQ ID NO: 751 1; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl), MYC inhibitor B (mRNA sequence shown in SEQ ID NO: 7513; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl), MYC inhibitor C (mRNA sequence shown in SEQ ID NO: 7515; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl) and MYC inhibitor

D (mRNA sequence shown in SEQ ID NO: 6621; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl). Cellular activity was measured 48 hours post-transfection with the use of WST-1 according to manufacturer's instructions (Roche, Indianapolis, IN). Absorbance readings were taken at 450 nm 4 hours after the addition of WST-1, and background-corrected results are shown in Table 47. The three highest concentrations of each of the inhibitors (MYC inhibitor A, MYC inhibitor B, MYC inhibitor C and MYC inhibitor D) reduced absorbance signal compared to the controls.

Table 47. Cellular Activity

Description	Amount of mRNA (nM)	WST-1 OD mean (450-690 nm)
MYC inhibitor A	0	0.45
	0.2	0.42
	0.7	0.09
	2	0.12
	6	0.05
MYC inhibitor B	0	0.67
	0.2	0.69
	0.7	0.24
	2	0.09
	6	0.05
MYC inhibitor C	0	0.73
	0.2	0.73
	0.7	0.34
	2	0.09
	6	0.04
MYC inhibitor D	0	0.74
	0.2	0.68
	0.7	0.32
	2	0.14
	6	0.07
mCherry	0	0.66
	0.2	0.66
	0.7	0.62
	2	0.60
	6	0.51
Non-translatable FIX	0	0.65
	0.2	0.65
	0.7	0.61
	2	0.62
	6	0.49

Wild-Type MYC	0	0.58
	0.2	0.51
	0.7	0.51
	2	0.51
	6	0.46

Example 56. *In Vivo* Expression of Modified mRNA

A. BALB/C nuce mice

[001117] BALB/c nude mice were injected intravenously with 0.1 mg/kg luciferase modified mRNA without a miR-122 binding site ("non-targeted mRNA"; mRNA sequence shown in SEQ ID NO: 7518; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl; fully modified with 5-methylcytidine and 1-methylpeudouridine) formulated in a lipid nanoparticle described in Table 48 or luciferase modified mRNA with a miR-122 binding site in the 3'UTR ("miR-122 targeted mRNA"; mRNA sequence shown in SEQ ID NO: 7519; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl; fully modified with 5-methylcytidine and 1-methylpeudouridine) formulated in a lipid nanoparticle described in Table 49.

Table 48. Lipid Nanoparticle for Non-targeted mRNA

LNP	Luciferase: non-targeted mRNA
Lipid	DLin-KC2-DMA
Lipid/RNA wt/wt	20
Mean size	73.3 nm PDI: 0.06

Table 49. Lipid Nanoparticle for Targeted mRNA

LNP	Luciferase: targeted mRNA
Lipid	DLin-KC2-DMA
Lipid/RNA wt/wt	20
Mean size	70.6 nm PDI: 0.08

[001118] 24 hours post-treatment, animals were anesthetized, injected with the luciferase substrate D-luciferin and the bioluminescence imaging (BLI) from living animals was evaluated in an IVIS imager 15 minutes later. Signals were obtained from animals injected with non-targeted mRNA and from miR-122 targeted mRNA, and presented in Table 50. The total light signal produced from livers of animals treated with miR 122

targeted mRNA is 29x lower than non-targeted mRNA, showing that the engineered element in the 3'UTR may inhibit protein expression in normal tissue.

Table 50. *In vivo* **expression of modified mRNA modulated by an engineered miR122 binding site**

Description	Luciferase signal from liver (photons/sec)
Non-targeted mRNA	7.9×10^7
miR-122 targted mRNA	2.7×10^6

B. BALB/c nude mice with hepatocellular carcinoma Hep3B cells

[001119] BALB/c nude mice were intrahepatically implanted with 2xl0 ^{6} hepatocellular carcinoma Hep3B cells and resulting orthotopic tumors allowed to grow for 24 days. Tumor-bearing mice were then intravenously injected with 0.1 mg/kg luciferase modified mRNA without a miR-122 binding site ("non-targeted mRNA"; mRNA sequence shown in SEQ ID NO: 7518; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl; fully modified with 5-methylcytidine and 1-methylpeudouridine) or luciferase modified mRNA with a miR-122 binding site in the 3'UTR ("miR-122 targeted mRNA"; mRNA sequence shown in SEQ ID NO: 7519; polyA tail of approximately 140 nucleotides not shown in sequence; 5' cap, Capl; fully modified with 5-methylcytidine and 1-methylpeudouridine) formulated in a lipid nanoparticle described in Table 45 (above). 24 hr post-treatment animals were anesthetized, injected with the luciferase substrate D-luciferin and bioluminescence imaging (BLI) from living animals was evaluated in an IVIS imager 20 minutes later. Signal from orthotopic tumors compared to adjacent normal liver was quantified, and miR-122-targeted mRNA systemically delivered via lipid nanoparticles achieved over 2-fold enrichment in tumor compared to normal liver.

Example 57. Modified nucleic acids with a mir-122 sequence

A. *HeLa Cells*

[001120] HeLa cells were seeded at a density of 15,000 per well in 100 ul cell culture medium (DMEM + 10%FBS). G-CSF mRNA having a miR-122 sequence in the 3'UTR (G-CSF miR122; mRNA sequence shown in SEQ ID NO: 7325; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap,Cap 1; fully modified with

5-methylcytosine and 1-methylpseudouridine) or G-CSF mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF seedless; mRNA sequence shown in SEQ ID NO: 7327; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) were transfected with 0.3ul per well of Lipofectamine 2000 at a concentration of 75 ng of mRNA per well in 96 well plates. The supernatant was collected between 16-18 hours after transfection and expression of G-CSF was measured by ELISA, and the results are shown in Table 51.

Table 51. G-CSF Expression in HeLa

Description	Protein Expression (ng/ml)
G-CSF miR122	292.1
G-CSF seedless	335.7

B. *Primary Human and Rat Hepatocytes*

[001121] Primary human or rat hepatocytes cells were seeded at a density of 350,000 cells per well in 500 ul cell culture medium *(InvitroGRO* CP and InVitroGRO HI Medium + 2.2% Torpedo Antibiotic Mix). G-CSF mRNA having a miR-122 sequence in the 3'UTR (G-CSF miR122; mRNA sequence shown in SEQ ID NO: 7520; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) or G-CSF mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF seedless; mRNA sequence shown in SEQ ID NO: 7521; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) were transfected with 1 ul per well of Lipofectamine 2000 at a concentration of 500 ng of mRNA per well in 24 well plates for the primary human hepatocytes and the primary rat hepatocytes. The supernatant was collected between 16-18 hours after transfection and expression of G-CSF was measured by ELISA, and the results are shown in Table 52. The mir-122 binding site sequence in the mRNA dampened the G-CSF protein expression in the primary hepatocytes.

Table 52. G-CSF Expression in Hepatocytes

Description	Primary Human Hepatocytes	Primary Rat Hepatocytes
	Protein Expression (ng/ml)	Protein Expression (ng/ml)
G-CSF miR122	116	26

G-CSF seedless	463	85

Example 58. Time Course of Modified nucleic acids with a mir-122 sequence

A. *HeLa Cells*

[001122] HeLa cells were seeded at a density of 17,000 per well in 100 ul cell culture medium (DMEM + 10%FBS). G-CSF mRNA without a miR-122 sequence in the 3'UTR (G-CSF; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7321; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7320; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 sequence in the 3'UTR (G-CSF miR122; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7325; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7322; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 seed sequence in the 3'UTR (G-CSF seed; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7326; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7323; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF seedless; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7327; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7324; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), Factor IX mRNA without a miR-122 sequence in the 3'UTR (FIX; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7329; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-

methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7328; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), Factor IX mRNA having a miR-122 sequence in the 3'UTR (FIX miR122; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7333; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7330; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), Factor IX mRNA having a miR-122 seed sequence in the 3'UTR (FIX seed; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7334; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7331; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) or Factor IX mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (FIX seedless; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7335; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7332; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) were transfected with 0.3 ul per well of Lipofectamine 2000 at a concentration of 75 ng of mRNA per well in 96 well plates. The supernatant was collected between 16-18 hours after transfection, expression of G-CSF or Factor IX was measured by ELISA, and the results are shown in Table 53.

Table 53. Expression in HeLa

Description	Protein Expression Mm 3'UTR (ng/ml)	Protein Expression Hs 3'UTR (ng/ml)
G-CSF	271.72	69.4
G-CSF miR122	305.36	68.8
G-CSF seed	209.5	98.0
G-CSF seedless	243.2	80.9
FIX	249.8	131.6
FIX mir122	204.6	55.4

FIX seed	290.05	127.6
FIX seedless	180.9	31.6

B. *Primary Human and Rat Hepatocytes*

[001123] Primary human or rat hepatocytes cells were seeded at a density of 350,000 cells per well in 500 ul cell culture medium *(InvitroGRO* CP and InVitroGRO HI Medium + 2.2% Torpedo Antibiotic). G-CSF mRNA without a miR-122 sequence in the 3'UTR (G-CSF; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7321; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7320; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 sequence in the 3'UTR (G-CSF miR122; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7325; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7322; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 seed sequence in the 3'UTR (G-CSF seed; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7326; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7323; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF seedless; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7327; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7324; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), Factor IX mRNA without a miR-122 sequence in the 3'UTR (FIX; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7329; polyA tail of approximately 140 nucleotides not shown in

sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7328; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), Factor IX mRNA having a miR-122 sequence in the 3'UTR (FIX miR122; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7333; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7330; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), Factor IX mRNA having a miR-122 seed sequence in the 3'UTR (FIX seed; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7334; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7331; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) or Factor IX mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (FIX seedless; mouse 3' UTR mRNA sequence shown in SEQ ID NO: 7335; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine; human 3'UTR mRNA sequence shown in SEQ ID NO: 7332; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) were transfected with 1 ul per well of Lipofectamine 2000 at a concentration of 500 ng per well in 24 well plates for the primary human hepatocytes and the primary rat hepatocytes. The supernatant was collected at 24 hours, 48 hours and 72 hours after transfection, expression of G-CSF and Factor IX was measured by ELISA, and the results are shown in Table 54. The mir-122 binding site sequence in the mRNA dampened the G-CSF and Factor IX protein expression in the primary hepatocytes.

Table 54. G-CSF Expression in Hepatocytes

Description	Time Point	Primary Human Hepatocytes	Primary Human Hepatocytes
		Protein Expression (ng/ml) Mm 3'UTR	Protein Expression (ng/ml) Hs 3'UTR

G-CSF	24 hours	43.9	84.9
	48 hours	18.8	100.4
	72 hours	5.7	21.3
G-CSF miR122	24 hours	6.9	24.0
	48 hours	.7	3.03
	72 hours	.12	.88
G-CSF seed	24 hours	48.5	115.8
	48 hours	25.6	96.4
	72 hours	8.2	19.2
G-CSF seedless	24 hours	31.7	113.1
	48 hours	11.7	92.9
	72 hours	3.4	18.9
FIX	24 hours	90.8	63.2
	48 hours	159.6	124.8
	72 hours	70.5	44.3
FIX mirl22	24 hours	11.8	15.9
	48 hours	5.0	4.4
	72 hours	1.0	.4
FIX seed	24 hours	77.2	60.2
	48 hours	115.0	63.0
	72 hours	41.7	20. 1
FIX seedless	24 hours	69.3	53.7
	48 hours	123.8	75.0
	72 hours	49.0	24.5

Example 59. Time Course of Modified nucleic acids with a mir-122 sequence in cancer cells

A. *Base Level of miR-122*

[001124] The base level of mir-122 in Human hepatocytes, rat hepatocytes, human hepatocellular carcinoma cells (Hep3B) and HeLa cells were determined by TAQMAN® analysis using the manufacturers protocol. The levels were normalized to U6 and the results are shown in Table 55.

Table 55. miR-122 Levels in Various Cell Types

Cell Type	miR-122 level (normalized to U6)
Human Hepatocytes	16.8
Rat Hepatocytes	10.9
Hep3B	0
HeLa	0

B. *Primary Human Hepatocytes and Hep3B Cells*

[001125] Primary human hepatocytes were seeded at a density of 50,000 cells per well in 100 ul cell culture medium *(InvitroGRO* CP and InVitroGRO HI Medium + 2.2% Torpedo Antibiotic Mix) and Hep3B cells were seeded at a density of 20,000 cells per

well in 100 ul cell culture medium MEM + 10%FBS. G-CSF mRNA without a miR-122 sequence in the 3'UTR (G-CSF; mRNA sequence shown in SEQ ID NO: 7320; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 sequence in the 3'UTR (G-CSF miR122; mRNA sequence shown in SEQ ID NO: 7322; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-122 seed sequence in the 3'UTR (G-CSF seed; mRNA sequence shown in SEQ ID NO: 7323; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) or G-CSF mRNA having a miR-122 sequence without the seed sequence in the 3'UTR (G-CSF seedless; mRNA sequence shown in SEQ ID NO: 7324; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) were transfected with 0.3 ul per well of Lipofectamine 2000 at a concentration of 75 ng of mRNA per well in 96 well plates for the primary human hepatocytes and the Hep3B cells. The supernatant was collected at 24 hours, 48 hours and 72 hours after transfection, expression of G-CSF was measured by ELISA, and the results are shown in Table 56. The mir-122 binding site sequence in the mRNA dampened the G-CSF protein expression in the primary human hepatocytes but not in the Hep3B cells.

Table 56. G-CSF Expression

Description	Time Point	Primary Human Hepatocytes	Hep3B
		Protein Expression (ng/ml) Hs 3'UTR	Protein Expression (ng/ml) Hs 3'UTR
G-CSF	24 hours	76	55
	48 hours	12	33
	72 hours	6	10
G-CSF miR122	24 hours	32	37
	48 hours	1	27
	72 hours	0	6
G-CSF seed	24 hours	75	39
	48 hours	11	28
	72 hours	4	6
G-CSF seedless	24 hours	79	49
	48 hours	15	35
	72 hours	6	9

Example 60. Time Course of Modified nucleic acids with a mir-142 3p sequence

A. *Base Level of miR-143 3p*

[001126] The base level of miR-142 3p in RAW264.7 cells and HeLa cells were determined by TAQMAN® analysis using the manufacturer's protocol. The levels were normalized to U6 and the results are shown in Table 57.

Table 57. miR-142 3p Levels in Various Cell Types

Cell Type	miR-122 level (normalized to U6)
Human Hepatocytes	16.8
Rat Hepatocytes	10.9
Hep3B	0
HeLa	0

B. *HeLa and RAW264.7 Cells*

[001127] HeLa cells were seeded at a density of 17,000 per well in 100 ul cell culture medium DMEM + 10%FBS and RAW264.7 cells were seeded at a density of 200,000 per well in 100 ul cell culture medium DMEM + 10%FBS. G-CSF mRNA without a miR-142 3p sequence in the 3'UTR (G-CSF; mRNA sequence shown in SEQ ID NO: 7522; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-142 3p sequence in the 3'UTR (G-CSF miR142 3p; mRNA sequence shown in SEQ ID NO: 7523; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-142 3p seed sequence in the 3'UTR (G-CSF seed; mRNA sequence shown in SEQ ID NO: 7524; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) or G-CSF mRNA having a miR-142 3p sequence without the seed sequence in the 3'UTR (G-CSF seedless; mRNA sequence shown in SEQ ID NO: 7525; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) were transfected with 0.3ul per well of Lipofectamine 2000 at a concentration of 75 ng of mRNA per well in 96 well plates for HeLa or with 1 ul per well of Lipofectamine 2000 at a concentration of 250 ng of mRNA per well in 24 well plates for RAW264.7 cells. The supernatant was collected 16-18 hours after transfection, expression of G-CSF was measured by ELISA, and the results

are shown in Table 58. miR-142 3p sites in G-CSF were shown to down-regulate G-CSF expression in RAW264.7 cells.

Table 58. Expression

Description	HeLa Protein Expression (ng/ml)	RAW264.7 Protein Expression (ng/ml)
G-CSF	243.5	124.8
G-CSF miR142 3p	309.1	42.8
G-CSF seed	259.8	148.1
G-CSF seedless	321.7	185.2

C. *Time Course in RAW264.7 Cells*

[001128] RAW264.7 cells were seeded at a density of 60,000 cells per well in 100 ul cell culture medium (DMEM + 10%FBS). G-CSF mRNA without a miR-142 3p sequence in the 3'UTR (G-CSF; mRNA sequence shown in SEQ ID NO: 7522; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-142 3p sequence in the 3'UTR (G-CSF miR142 3p; mRNA sequence shown in SEQ ID NO: 7523; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-142 3p seed sequence in the 3'UTR (G-CSF seed; mRNA sequence shown in SEQ ID NO: 7524; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) or G-CSF mRNA having a miR-142 3p sequence without the seed sequence in the 3'UTR (G-CSF seedless; mRNA sequence shown in SEQ ID NO: 7525; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) were transfected with 0.3 ul per well of Lipofectamine 2000 at a concentration of 75 ng of mRNA per well in 96 well plates. The supernatant was collected at 24 hours, 48 hours and 72 hours after transfection, expression of G-CSF was measured by ELISA, and the results are shown in Table 59. The mir-142 3p binding site sequence in the mRNA showed a strong suppression of G-CSF expression in RAW264.7 cells over time.

Table 59. G-CSF Expression

Description	Time Point	RAW264.7 Cells
		Protein Expression (ng/ml)
G-CSF	24 hours	133.5

	48 hours	69.7
	72 hours	2.1
G-CSF miR142 3p	24 hours	60.1
	48 hours	9.2
	72 hours	.3
G-CSF seed	24 hours	244.9
	48 hours	68.9
	72 hours	2.3
G-CSF seedless	24 hours	250.2
	48 hours	95.9
	72 hours	3.0

D. *miR-142 3p in PBMC*

[001129] Peripheral blood mononuclear cells (PBMCs) were seeded at a density of 150,000 cells per well in 100 ul cell culture medium (Opti-MEM and after transfection add 10%FBS). G-CSF mRNA having a miR-142 3p sequence in the 3'UTR (G-CSF miR142 3p; mRNA sequence shown in SEQ ID NO: 7523; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, Capl; fully modified with 5-methylcytosine and 1-methylpseudouridine), G-CSF mRNA having a miR-142 3p seed sequence in the 3'UTR (G-CSF seed; mRNA sequence shown in SEQ ID NO: 7524; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) or G-CSF mRNA having a miR-142 3p sequence without the seed sequence in the 3'UTR (G-CSF seedless; mRNA sequence shown in SEQ ID NO: 7525; polyA tail of approximately 140 nucleotides not shown in sequence; 5'cap, capl; fully modified with 5-methylcytosine and 1-methylpseudouridine) were transfected in triplicate with 0.4 ul per well of Lipofectamine 2000 at a concentration of 500 ng of mRNA per well in 96 well plates for 2 or 3 donors. The supernatant was collected at 24 hours after transfection and the expression of G-CSF was measured by ELISA. The results for the 2 donors are shown in Table 60 and the results for the 3 donors are shown in Table 61. The mir-142 3p binding site sequence in the mRNA was shown to down regulate G-CSF expression in human PBMC.

Table 60. Expression PBMC (2 donors)

Description	Protein Expression (ng/ml)
G-CSF miR142 3p	5.09
G-CSF seed	10.06
G-CSF seedless	9.38

Table 61. Expression PBMC (3 donors)

Description	Protein Expression (ng/ml)
G-CSF miR142 3p	7.48
G-CSF seed	13.40
G-CSF seedless	13.98

OTHER EMBODIMENTS

[001130] It is to be understood that the words which have been used are words of description rather than limitation, and that changes may be made within the purview of the appended claims without departing from the true scope and spirit of the invention in its broader aspects.

[001131] While the present invention has been described at some length and with some particularity with respect to the several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope of the invention.

[001132] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, section headings, the materials, methods, and examples are illustrative only and not intended to be limiting.

Claims

We claim:

1. An isolated synthetic signal-sensor polynucleotide, wherein said isolated synthetic signal-sensor polynucleotide comprises an mRNA which encodes an oncology-related polypeptide of interest and one or more sensor sequences selected from the group consisting of any of SEQ ID NOs: 3529-4549, SEQ ID NOs: 5571-6591 and functional variants thereof.

2. The isolated synthetic signal-sensor polynucleotide of claim 1 wherein the oncology-related polypeptide of interest is selected from the group consisting of SEQ ID NOs: 1321-2487, 661 1-6616, 7355-7361, 7490, 7492, 7493, 7512, 7514, 7516 and 7517.

3. The isolated synthetic signal-sensor polynucleotide of claim 1 wherein the mRNA comprises at least an open reading frame of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 2488-2496, 6617-6621, 7348-7354, 7362-7489, 7491, 7494, 7506, 751 1 and 7513.

4. The isolated synthetic signal-sensor polynucleotide of claim 3, wherein the open reading frame is codon optimized.

5. The isolated synthetic signal-sensor polynucleotide of claim 1, wherein the mRNA comprises two stop codons.

6. The isolated synthetic signal-sensor isolated polynucleotide of claim 1, wherein the mRNA comprises a first stop codon "TGA" and a second stop codon selected from the group consisting of "TAA," "TGA" and "TAG."

7. The isolated synthetic signal-sensor polynucleotide of claim 1, wherein the mRNA has a 3' tailing sequence of linked nucleosides selected from the group

consisting of a poly-A tail of at least 140 nucleotides, a triple helix, and a poly A-G quartet.

8. The isolated synthetic signal-sensor polynucleotide of claim 1, wherein the mRNA comprises at least one 5'terminal cap selected from the group consisting of CapO, Capl, ARCA, inosine, Nl-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

9. The isolated synthetic signal-sensor polynucleotide of claim 1, where the isolated synthetic signal-sensor polynucleotide is substantially purified.

10. The isolated synthetic signal-sensor polynucleotide of claim 1, wherein the isolated synthetic signal-sensor polynucleotide comprises at least one chemical modification.

11. The isolated synthetic signal-sensor polynucleotide of claim 10, wherein the at least one chemical modification is 1-methylpseudouridine.

12. The isolated synthetic signal-sensor polynucleotide of claim 11, further comprising the chemical modification 5-methylcytidine.

13. The isolated synthetic signal-sensor polynucleotide of claim 1, where the isolated synthetic signal-sensor polynucleotide comprises at least two chemical modifications.

14. The isolated synthetic signal-sensor polynucleotide of claim 13, wherein the modifications are located on one or more of a nucleoside and/or the backbone of said nucleotides.

15. The isolated synthetic signal-sensor polynucleotide of claim 13, where the modifications are located on both a nucleoside and a backbone linkage.

16. The isolated synthetic signal-sensor polynucleotide of claim 13, where the modifications are located on the backbone linkage.

17. The isolated synthetic signal-sensor polynucleotide of claim 1, wherein the isolated signal-sensor polynucleotide is codon optimized.

18. The isolated synthetic signal-sensor polynucleotide of claim 1, wherein the isolated signal-sensor polynucleotide is formulated.

19. The isolated synthetic signal-sensor polynucleotide of claim 1 wherein the polypeptide of interest is a factor modulating the affinity between HIF subunits and/or HIF-dependent gene expression.

20. The isolated synthetic signal-sensor polynucleotide of claim 19 wherein the HIF subunits are selected from the group consisting of SEQ ID NO: 661 1-6616.

21. The isolated synthetic signal-sensor polynucleotide of claim 1 wherein the isolated synthetic signal-sensor polynucleotide comprises at least one translation enhancer element.

22. An isolated synthetic signal-sensor polynucleotide comprising:
    (a) a first region of linked nucleosides, said first region encoding an oncology-related polypeptide of interest selected from the group consisting of SEQ ID NOs: 1321-2487, 661 1-6616 and 7355-7361, 7490, 7492, 7493, 7512, 7514, 7516 and 7517;
    (b) a first flanking region located 5' relative to said first region comprising;

(i) a sequence of linked nucleosides selected from the group consisting of the native 5' untranslated region (UTR) of any of the nucleic acids that encode any of SEQ ID NOs: 1321-2487, 661 1-6616, 7355-7361, 7490, 7492, 7493, 7512, 7514, 7516, 7517, SEQ ID NO: 1-4 and functional variants thereof;

(c) a second flanking region located 3' relative to said first region comprising;

(i') a sequence of linked nucleosides selected from the group consisting of the native 3' UTR of any of the nucleic acids that encode any of SEQ ID NOs: 1321-2487, 661 1-6616, 7355-7361, 7490, 7492, 7493, 7512, 7514, 7516, 7517, SEQ ID NO: 5-21 and functional variants thereof;

(ü ') one or more sensor sequences located selected from the group consisting of the any of SEQ ID NOs: 3529-4549, SEQ ID NOs: 5571-6591 and functional variants thereof; and

(iii') a 3' tailing sequence of linked nucleosides.

23. The isolated synthetic signal-sensor polynucleotide of claim 22 wherein the first region of linked nucleosides comprises at least an open reading frame of a nucleic acid sequence, wherein the nucleic acid sequence is selected from the group consisting of SEQ ID NOs: 2488-2496, 6617-6621, 7348-7354, 7362-7489, 7491, 7494, 7506, 751 1 and 7513.

24. The isolated synthetic signal-sensor polynucleotide of claim 23, wherein the open reading frame is codon optimized.

25. The isolated synthetic signal-sensor polynucleotide of claim 22, wherein the first region comprises two stop codons.

26. The isolated synthetic signal-sensor isolated polynucleotide of claim 22, wherein the first region comprises a first stop codon "TGA" and a second stop codon selected from the group consisting of "TAA," "TGA" and "TAG."

27. The isolated synthetic signal-sensor polynucleotide of claim 22, wherein the 3' tailing sequence of linked nucleosides is selected from the group consisting of a poly-A tail of at least 140 nucleotides, a triple helix, and a poly A-G quartet.

28. The isolated synthetic signal-sensor polynucleotide of claim 22, wherein the first flanking region further comprises at least one 5'terminal cap.

29. The isolated synthetic signal-sensor polynucleotide of claim 28, wherein the at least one 5' terminal cap is selected from the group consisting of CapO, Capl, ARCA, inosine, Nl-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

30. The isolated synthetic signal-sensor polynucleotide of claim 28 where the isolated signal-sensor polynucleotide is substantially purified.

31. The isolated synthetic signal-sensor polynucleotide of claim 22, wherein the isolated synthetic signal-sensor polynucleotide comprises at least one chemical modification.

32. The isolated synthetic signal-sensor polynucleotide of claim 31, wherein the at least one chemical modification is 1-methylpseudouridine.

33. The isolated synthetic signal-sensor polynucleotide of claim 32, further comprising the chemical modification 5-methylcytidine.

34. The isolated synthetic signal-sensor polynucleotide of claim 22, comprising at least two chemical modifications in the first region.

35. The isolated synthetic signal-sensor polynucleotide of claim 34, wherein the modifications are located on one or more of a nucleoside and/or the backbone of said nucleotides.

36. The isolated synthetic signal-sensor polynucleotide of claim 34, where the modifications are located on both a nucleoside and a backbone linkage.

37. The isolated synthetic signal-sensor polynucleotide of claim 34, where the modifications are located on the backbone linkage.

38. The isolated synthetic signal-sensor polynucleotide of claim 22, where the isolated signal-sensor polynucleotide is codon optimized.

39. The isolated synthetic signal-sensor polynucleotide of claim 38, wherein the first region of linked nucleosides is codon optimized.

40. The isolated synthetic signal-sensor polynucleotide of claim 22, wherein the isolated signal-sensor polynucleotide is formulated.

41. A method of treating a disease, disorder and/or condition in a subject in need thereof by increasing the level of an oncology-related polypeptide of interest comprising administering to said subject an isolated synthetic signal-sensor polynucleotide encoding said oncology-related polypeptide.

42. A method of reducing, eliminating or preventing tumor growth in a subject in need thereof by increasing the level of an oncology-related polypeptide of interest comprising administering to said subject an isolated synthetic signal-sensor polynucleotide encoding said oncology-related polypeptide.

43. A method of reducing and/or ameliorating at least one symptom of cancer in a subject need thereof by increasing the level of an oncology-related polypeptide of

interest comprising administering to said subject an isolated synthetic signal-sensor polynucleotide encoding said oncology-related polypeptide.

44. The method of any of claims 41-43 wherein the disease, disorder and/or condition is selected from the group consisting of adrenal cortical cancer, advanced cancer, anal cancer, aplastic anemia, bileduct cancer, bladder cancer, bone cancer, bone metastasis, brain tumors, brain cancer, breast cancer, childhood cancer, cancer of unknown primary origin, Castleman disease, cervical cancer, colon/rectal cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, gestational trophoblastic disease, Hodgkin disease, Kaposi sarcoma, renal cell carcinoma, laryngeal and hypopharyngeal cancer, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myelomonocytic leukemia, liver cancer, hepatocellular carcinoma (HCC), non-small cell lung cancer, small cell lung cancer, lung carcinoid tumor, lymphoma of the skin, malignant mesothelioma, multiple myeloma, myelodysplasia syndrome, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma in adult soft tissue, basal and squamous cell skin cancer, melanoma, small intestine cancer, stomach cancer, testicular cancer, throat cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, Wilms tumor and secondary cancers caused by cancer treatment.

45. The method of claim 44 wherein the tumor growth is results from a disease, disorder and/or condition selected from the group consisting of adrenal cortical cancer, advanced cancer, anal cancer, aplastic anemia, bileduct cancer, bladder cancer, bone cancer, bone metastasis, brain tumors, brain cancer, breast cancer, childhood cancer, cancer of unknown primary origin, Castleman disease, cervical

cancer, colon/rectal cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, gestational trophoblastic disease, Hodgkin disease, Kaposi sarcoma, renal cell carcinoma, laryngeal and hypopharyngeal cancer, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myelomonocytic leukemia, liver cancer, hepatocellular carcinoma (HCC), non-small cell lung cancer, small cell lung cancer, lung carcinoid tumor, lymphoma of the skin, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma in adult soft tissue, basal and squamous cell skin cancer, melanoma, small intestine cancer, stomach cancer, testicular cancer, throat cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, Wilms tumor and secondary cancers caused by cancer treatment.

46. The method of any of claims 41-43 wherein the administration of the isolated synthetic signal-sensor polynucleotide reduces the number of cancer cells, eliminates cancer cells, prevents an increase in cancer cells and/or alleviates the symptoms of cancer in a subject.

47. The method of claim 43 wherein the at least one symptom of cancer is selected from the group consisting of weakness, aches and pains, fever, fatigue, weight loss, blood clots, increased blood calcium levels, low white blood cell count, short of breath, dizziness, headaches, hyperpigmentation, jaundice, erthema, pruritis, excessive hair growth, change in bowel habits, change in bladder function, long-lasting sores, white patches inside the mouth, white spots on the tongue, unusual bleeding or discharge, thickening or lump on parts of the body, indigestion,

trouble swallowing, changes in warts or moles, change in new skin and nagging cough and hoarseness.

48. The methods of any of claims 41-43, wherein the isolated synthetic signal-sensor polynucleotide is formulated.

49. The method of claim 48, wherein the isolated synthetic signal-sensor polynucleotide is administered at a total daily dose of between 0.001 ug and 150 ug-

50. The method of claim 49, wherein administration is by injection, topical administration, ophthalmic administration or intranasal administration.

51. The method of claim 50, wherein administration is by injection and said injection is selected from the group consisting of intradermal, subcutaneous and intramuscular.

52. The method of claim 50, wherein administration is topical administration and said topical administration is selected from the group consisting of cream, lotion, ointment, gel, spray, solution and the like.

53. A method of preferentially inducing cell death in cancer cells in a tissue or organ, comprising

(a) contacting said tissue or organ with an isolated synthetic signal-sensor polynucleotide, wherein said isolated synthetic signal-sensor polynucleotide encodes

(i) an oncology-related polypeptide whose expression triggers apoptosis or cell death, and

(ii) at least one microRNA binding site of a microRNA, where the expression of said microRNA in the cancer cell is lower than the expression of said microRNA in normal, non cancerous cells.

FIGURE 1

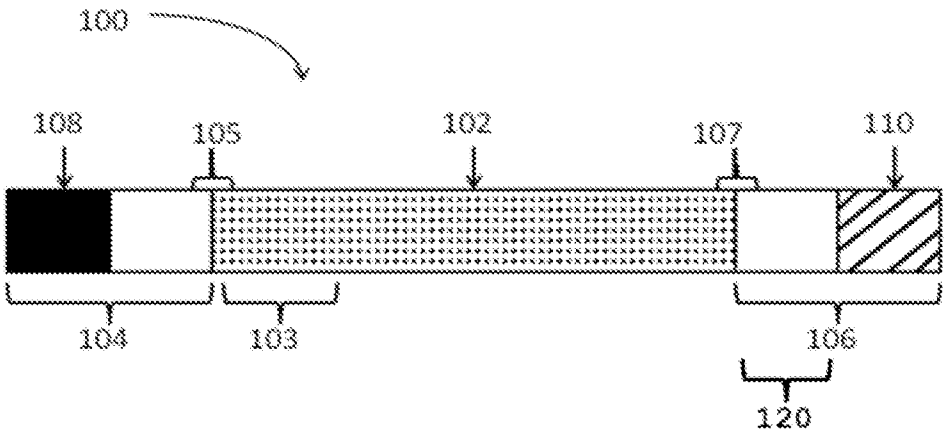


FIGURE 2

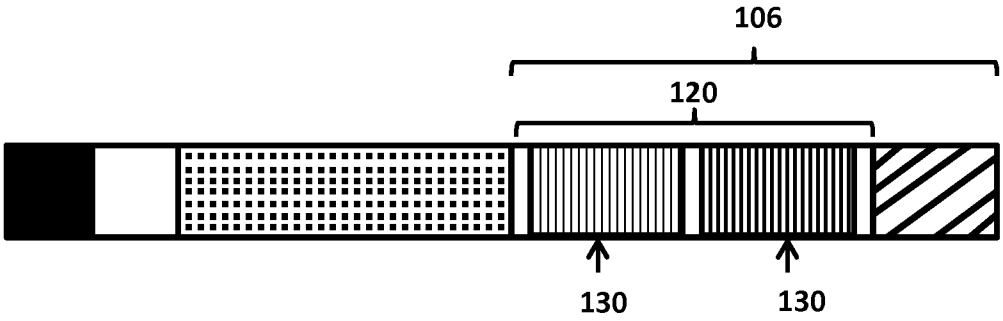
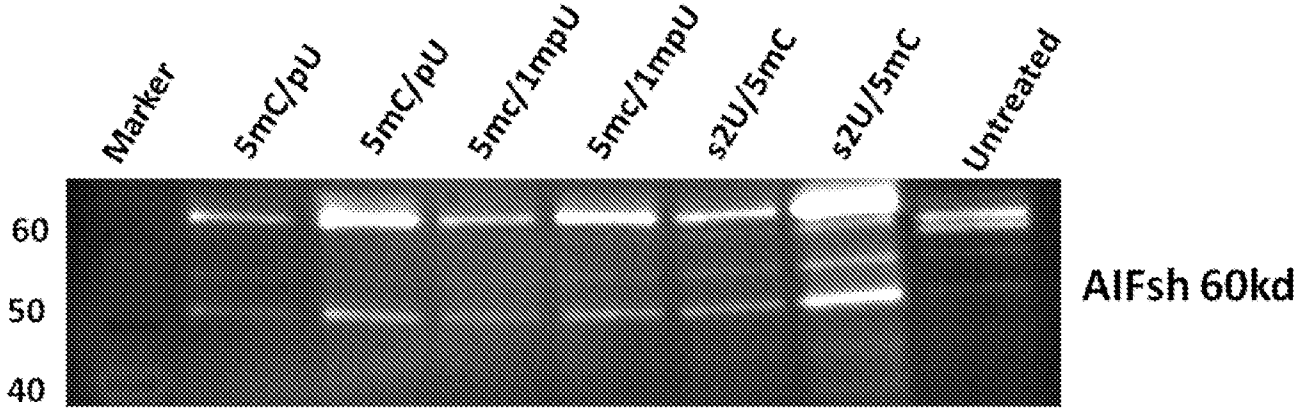


FIGURE 3

A.


Marker
5mC/pU
5mC/pU
5mc/1mpU
5mc/1mpU
s2U/5mC
s2U/5mC
Untreated
60
50
40
AIFsh 60kd

B.

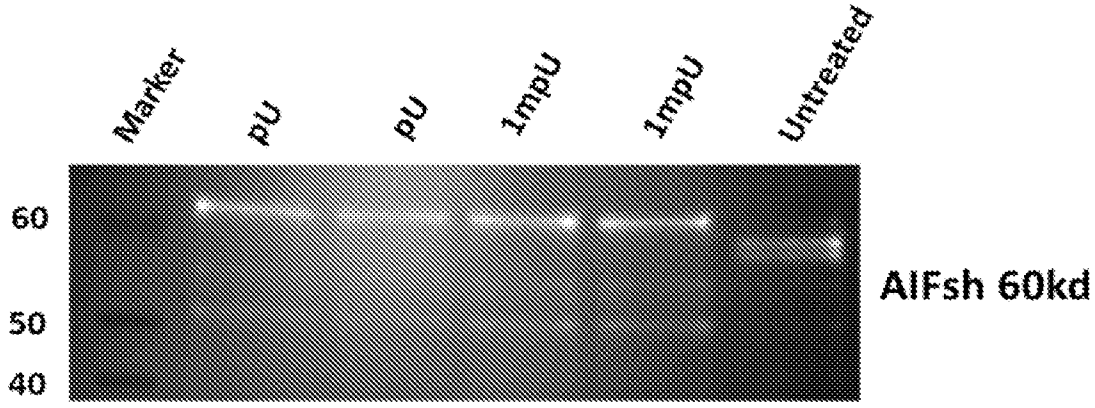
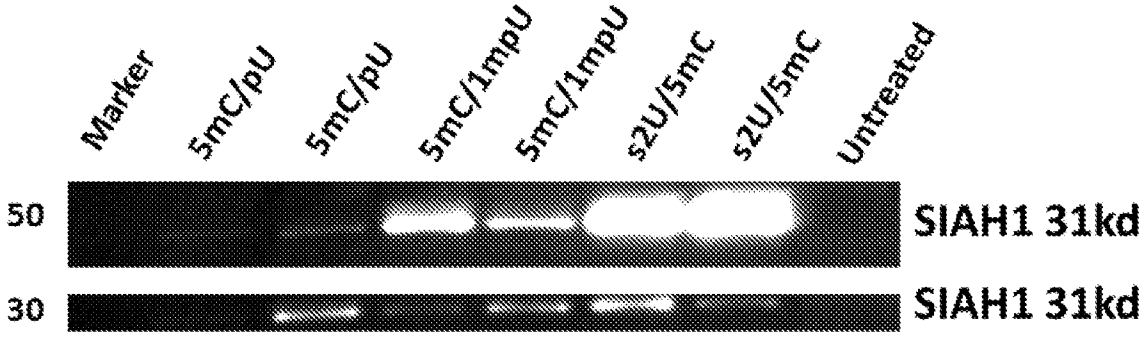

Marker
pU
pU
1mpU
1mpU
Untreated
60
50
40
AIFsh 60kd

FIGURE 4

A.


Marker
5mC/pU
5mC/pU
5mC/1mpU
5mC/1mpU
s2U/5mC
s2U/5mC
Untreated
50
SIAH1 31kd
30
SIAH1 31kd

B.

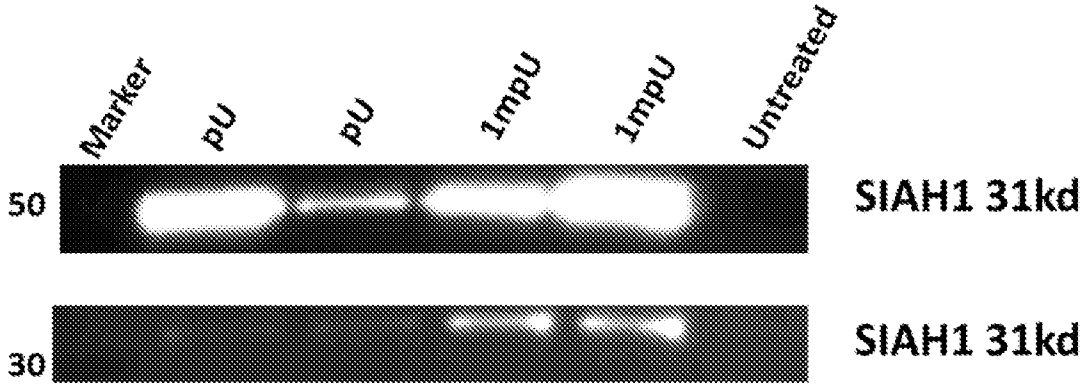
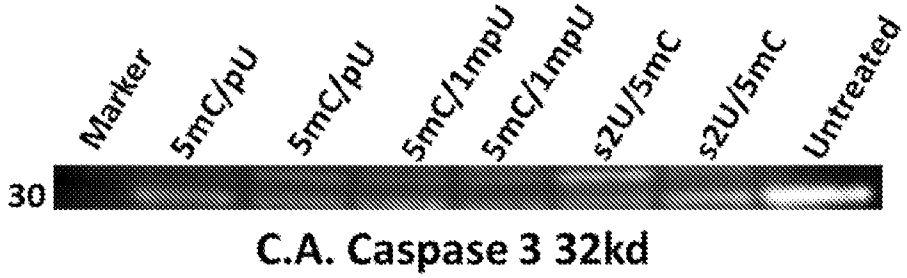

Marker
pU
pU
1mpU
1mpU
Untreated
50
SIAH1 31kd
30
SIAH1 31kd

FIGURE 5

A.


Marker
5mC/pU
5mC/pU
5mC/1mpU
5mC/1mpU
s2U/5mC
s2U/5mC
Untreated
30
C.A. Caspase 3 32kd

B.

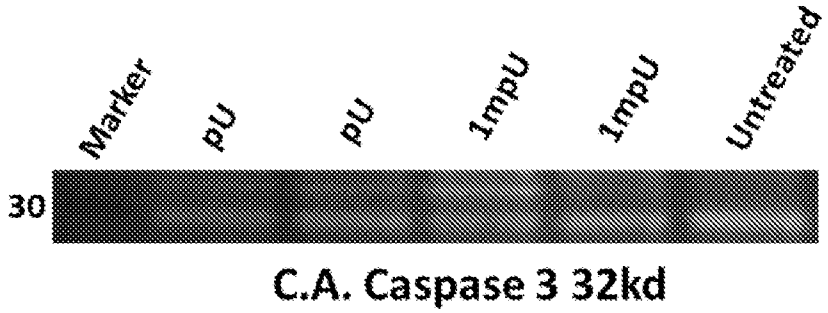
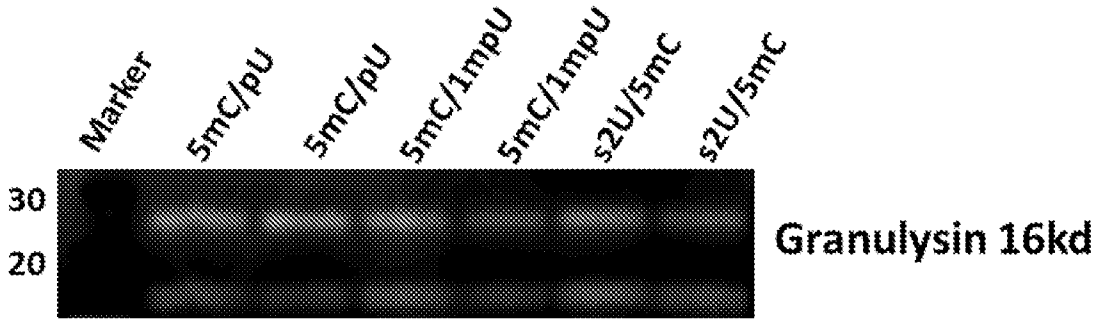

Marker
pU
pU
1mpU
1mpU
Untreated
30
C.A. Caspase 3 32kd

FIGURE 6

A.


Marker
5mC/pU
5mC/pU
5mC/1mpU
5mC/1mpU
s2U/5mC
s2U/5mC
30
20
Granulysin 16kd

B.

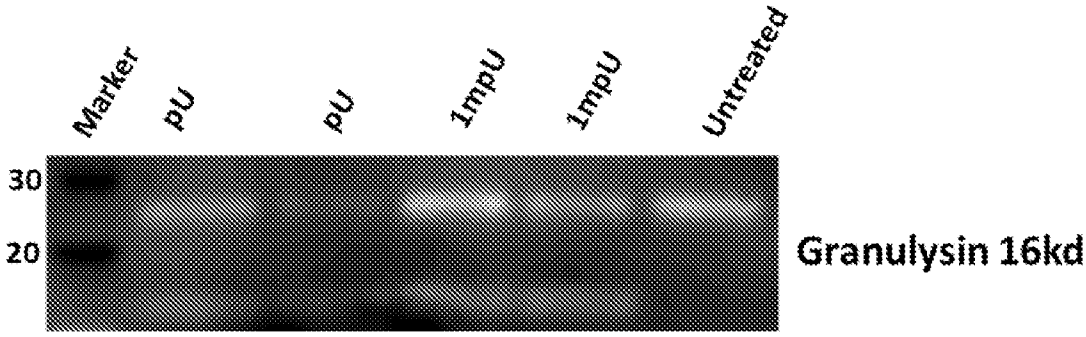
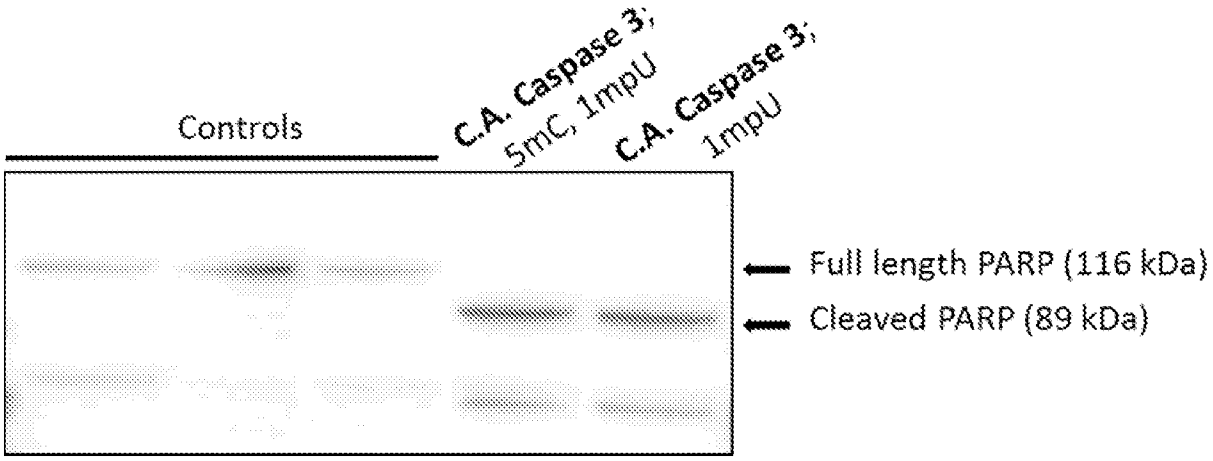
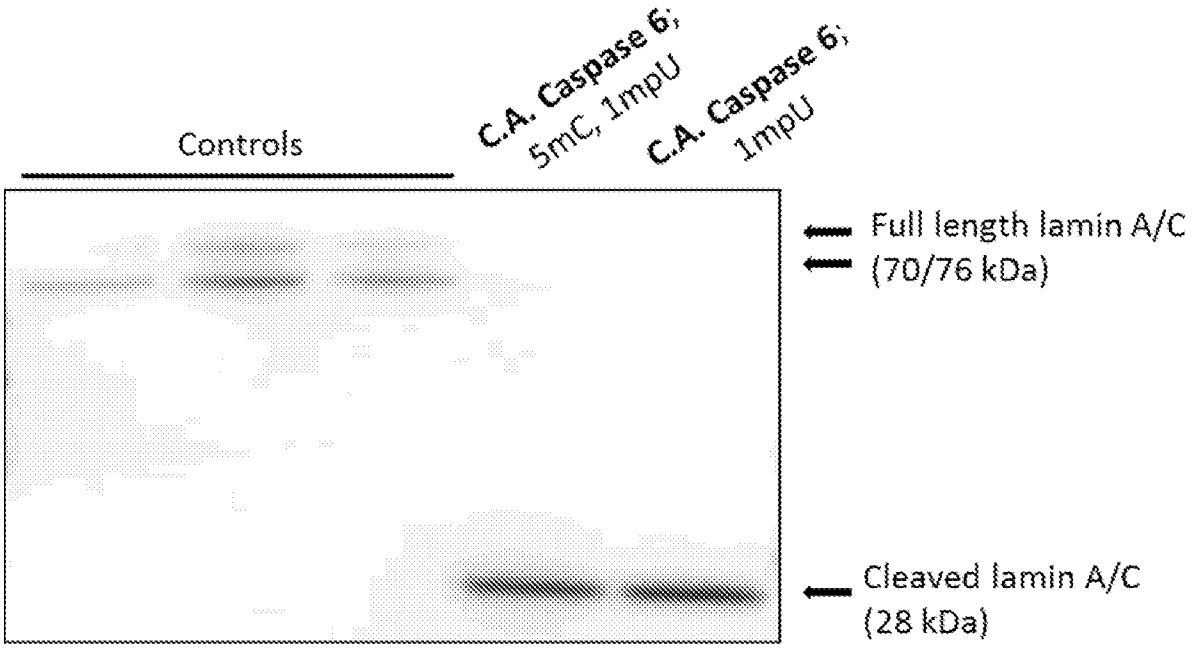

Marker
pU
pU
1mpU
1mpU
Untreated
30
20
Granulysin 16kd

Figure 7

A.


Controls
C.A. Caspase 3; 5mC, 1mpU
C.A. Caspase 3; 1mpU
Full length PARP (116 kDa)
Cleaved PARP (89 kDa)

B.


Controls
C.A. Caspase 6; 5mC, 1mpU
C.A. Caspase 6; 1mpU
Full length lamin A/C (70/76 kDa)
Cleaved lamin A/C (28 kDa)