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- (71) Applicant: BOSTON BIOMEDICAL, INC. [US/US];
640 Memorial Drive, Cambridge, MA 02139 (US).
- (72) Inventors: LI, Chiang, J.; 8 Museum Way, Cambridge,
MA 02141 (US). SUN, Xiangao; 121 Bond Road, Chest-
nut Hill, MA 02467 (US). YAMAKAWA, Erina-Koga;
1501 Beacon Street, #506, Brookline, MA 02446 (US).
- (74) Agents: SOUTHGATE, Christopher, D. et al.; Boston
Biomedical, Inc., Room 550, 640 Memorial Drive, Cam-
bridge, MA 02139 (US).
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- (54) Title: ASYMMETRIC INTERFERING RNAs, AND COMPOSITIONS, USE, OR PREPARATION THEREOF

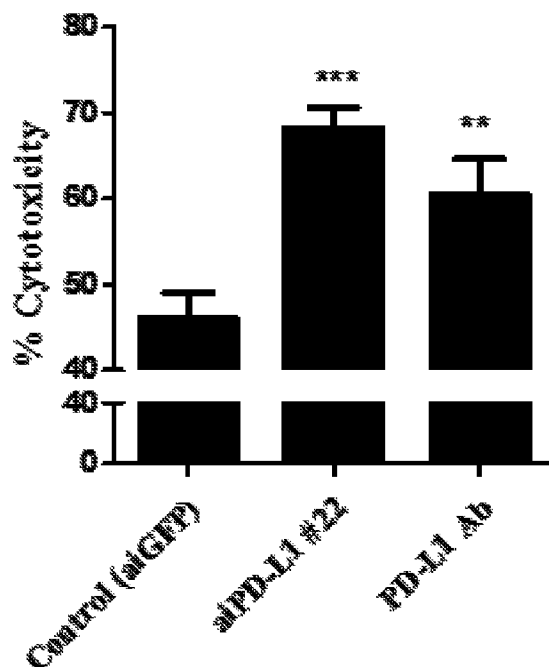


FIG. 5

(57) Abstract: The present teachings provide RNA molecules, including asymmetric interfering RNAs (aiRNAs), that are capable of reducing or inhibiting cancer cell's immune-evasion mechanisms by modulating the expression of immune checkpoint proteins; compositions thereof; and methods of using thereof. For example, one of the RNA molecules includes an antisense strand and a sense strand, where the antisense strand consists of 19-23 nucleotides, the sense strand consists of 14-17 nucleotides, where the sense strand is complementary to the antisense strand; and where the antisense strand comprises a sequence that is complementary to a target nucleotide sequence in an mRNA that encodes a ligand to PD-1.



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ASYMMETRIC INTERFERING RNAs, AND COMPOSITIONS, USE, OR PREPARATION THEREOF

[0001] Immuno-oncology is a promising new area for cancer therapeutics. The immune system is capable of exquisite adaptation and selective targeting, a process that is now being harnessed and directed toward advanced cancer. Therapies in this field manipulate the immune response against cancer in a number of different ways. Vaccines have been developed with the goal of priming the cellular and humoral immune response toward specific cancer antigens, much in the same way as vaccines for microbiological diseases would do. Other therapies target the specific immune evasion mechanisms that cancer cells use to avoid detection by the host immune system. These evasion mechanisms are the “checkpoints” of the immune system; specific cell-surface molecules that prevent the immune effectors from killing those cells that express them.

[0002] Recent clinical success with antibodies targeting programmed cell death-1 receptor (PD-1) and its ligands (PD-L1, PD-L2) has validated the general concept that cancer cells can hijack immune checkpoint genes to subvert endogenous anticancer surveillance by the immune system. For example, ipilimumab, first approved in the United States in 2011, targets cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4); while nivolumab and pembrolizumab, both of which were first approved in the United States in 2014, target PD-1. Despite the initial success shown by these immune checkpoint inhibitor antibodies, immune-related adverse, sometimes lethal, events have been observed which casts a significant doubt about their long term use (see, for example, Johnson *et al.* (2016). "Fulminant Myocarditis with Combination Immune Checkpoint Blockade." *New England Journal of Medicine* 375(18): 1749-1755). Thus, there remains an unmet need for novel safe and effective anticancer compounds, compositions, and uses thereof in treating cancer, and specifically for agents with activity against immune evasion mechanisms adopted by cancer cells.

[0003] The present teachings address such needs by providing PD-L1-specific asymmetric interfering RNAs (aiRNAs) that prevents signaling by the immune checkpoint molecule, PD-1. In certain embodiments, the aiRNAs are highly effective at silencing PD-L1 expression in tumors. In certain embodiments, the suppression of PD-L1 expression by tumor cells prevents the activation of the PD-1 immune checkpoint pathway in T cells which is in large part responsible for the down

regulation of tumor cell-specific T cell cytotoxicity and the concurrent breakdown of the immune surveillance for oncogenic cells in cancer patients.

[0004] In a first aspect, the present teachings provide an RNA molecule comprising an antisense strand comprising 5'-terminal and 3'-terminal nucleotides that are 17, 18, 19, 20, or 21 nucleotides apart, and a sense strand comprising a 5'-terminal nucleotide that is complementary to a nucleotide of the antisense strand other than its 3'-terminal nucleotide and a 3'-terminal nucleotide that is complementary to a nucleotide of the antisense strand, wherein at least 14 nucleotides of the antisense strand are complementary with the sense strand, and wherein at least 14 nucleotides of the antisense strand are colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.

[0005] In certain embodiments, the at least 14 nucleotides of the RNA molecule's antisense strand are colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163 or 164.

[0006] In certain embodiments, the RNA molecule's antisense strand comprises the nucleotide sequence of SEQ ID NO. 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91 or 92.

[0007] In certain embodiments, the RNA molecule's antisense strand comprises at least 7 nucleotides that are colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137 or 138.

[0008] In certain embodiments, the at least 7 of the at least 14 nucleotides of the RNA molecule's antisense strand are contiguous and colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.

[0009] In certain embodiments, the RNA molecule's sense strand comprises at least 14 nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.

[0010] In certain embodiments, the RNA molecule's sense strand comprises at least 14 nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163 or 164.

[0011] In certain embodiments, the RNA molecule's sense strand comprises a nucleotide sequence chosen from SEQ ID NO. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46.

[0012] In certain embodiments, 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 nucleotides of the RNA molecule's antisense strand are not contiguous with the corresponding colinear complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.

[0013] In certain embodiments, 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 nucleotides of the RNA molecule's antisense strand are not complementary to the corresponding colinear nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.

[0014] In certain embodiments, at least 14 nucleotides of the RNA molecule's sense strand are contiguous and colinear with the corresponding complementary nucleotides of the antisense strand.

[0015] In certain embodiments, the 5'-terminal nucleotide of the RNA molecule's sense strand is complementary to the first, second or third nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand.

[0016] In certain embodiments, the 3'-terminal nucleotide of the RNA molecule's sense strand is complementary to the first, second or third nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand.

[0017] In certain embodiments, the 5'-terminal and 3'-terminal nucleotides of the RNA molecule's sense strand are 13 nucleotides apart.

[0018] In certain embodiments, 19, 20 or 21 nucleotides of the RNA molecule's antisense strand are colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.

[0019] In certain embodiments, the nucleotide sequence of the RNA molecule's antisense strand that is colinear with the corresponding complementary nucleotides of the target nucleotide sequence has a GC content of about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34% or about 35%.

[0020] In certain embodiments, the nucleotide sequence of the RNA molecule's antisense strand that is colinear with the corresponding complementary nucleotides of the target nucleotide sequence has a GC content of about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43% or about 44%.

[0021] In certain embodiments, the nucleotide sequence of the RNA molecule's antisense strand that is colinear with the corresponding complementary nucleotides of the target nucleotide sequence has a GC content of about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57% or about 58%.

[0022] In certain embodiments, the nucleotide sequence of the RNA molecule's antisense strand that is colinear with the corresponding complementary nucleotides of the target nucleotide sequence has a GC content of about 33%.

[0023] In certain embodiments, the present teachings provide an RNA molecule chosen from aiRNAs 1-46.

[0024] In certain embodiments, either the RNA molecule's sense or antisense strand comprises at least one modified nucleotide or its analogue.

[0025] In certain embodiments, a 2'-OH group of the at least one modified ribonucleotide or its analogue is replaced by H or a 2'-O-methyl group.

[0026] In certain embodiments, the at least one modified nucleotide or its analogue is a sugar-, backbone-, and/or base-modified ribonucleotide. In certain embodiments, the backbone-modified ribonucleotide comprises a modification in a phosphodiester linkage with another ribonucleotide, for example, to comprise a nitrogen or a sulfur heteroatom. In certain embodiments, the at least one modified nucleotide or its analogue comprises a phosphothioate group, an inosine or a tritylated base. In certain embodiments, the at least one modified nucleotide or its analogue is a sugar-modified ribonucleotide, wherein a 2'-OH group is replaced by H, OR, R, halo, SH, SR,

NH₂, NHR, NR₂, or CN, and wherein each R is independently C1-C6 alkyl, alkenyl or alkynyl, and halo is F, Cl, Br, or I.

[0027] In certain embodiments, the 5'-terminal nucleotide and the first nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand comprise an "AA" motif, a "UU" motif, a "CC" motif, an "AU" motif, an "AC" motif, a "UA" motif, a "UC" motif, or a "CA" motif.

[0028] In certain embodiments, the disclosed RNA molecule comprises a deoxyribonucleotide.

[0029] In certain embodiments, the first nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand is not dT.

[0030] In certain embodiments, the silencing of a PD-L1 expressed nucleotide sequence by the RNA molecule is more effective than an anti-PD-L1 antibody. In certain embodiments, the RNA molecule is more effective than an anti-PD-L1 antibody at enhancing tumor-specific T cell cytotoxicity.

[0031] In certain embodiments, the administration of the RNA molecule into a subject in need thereof does not induce an interferon response.

[0032] In certain embodiments, disclosed is a composition that comprises one or more of the RNA molecules. In certain embodiments, the composition can be a nanoparticle composition.

[0033] In certain embodiments, disclosed is a kit that comprises one or more of the RNA molecules or compositions comprising one or more of the RNA molecules.

[0034] In certain embodiments, disclosed is an expression vector that comprises a nucleic acid sequence encoding the RNA molecule. The expression vector can be a viral, a eukaryotic, or a bacterial expression vector.

[0035] In certain embodiments, provided is an isolated cell that comprises the expression vector encoding the RNA molecule or the RNA molecule. The cell can be a mammalian, avian, insect, yeast or bacterial cell.

[0036] In a second aspect, a method is disclosed for treating a disease or condition comprising administering a therapeutically effective amount of the RNA molecule to a subject in need thereof.

[0037] In a third aspect, a method is disclosed for treating, preventing, or ameliorating cancer in a subject comprising administering a therapeutically effective amount of the RNA molecule to

a subject in need thereof. In certain embodiments, the cancer is an AIDS-Related cancer, a breast cancer, a cancer of the digestive/gastrointestinal tract, an endocrine and neuroendocrine cancer, a cancer of the eye, a genitourinary cancer, a germ cell cancer, a gynecologic cancer, a head and neck cancer, a hematologic cancer, a musculoskeletal cancer, a neurologic cancer, a respiratory/thoracic cancer, a skin cancer, a childhood cancer or a cancer of unknown primary. The RNA molecule can be administered systemically or locally. The cancer can be metastatic, recurrent or resistant to chemotherapy and/or radiation.

BRIEF DESCRIPTION OF DRAWINGS

[0038] Without being limited to any particular theory or analysis and with respect to the exemplified RNA molecules, FIG. 1A aligns the sense strands (SEQ ID NOs. 1-46) and antisense strands (SEQ ID NOs. 47-92) with exemplary PD-L1 target mRNA sequences (SEQ ID NOs. 140-164); depicts the length of the sense and antisense strands, the number and % GC content of nucleotides in the antisense strand that are colinear with the corresponding complementary nucleotides of the PD-L1 mRNA sequence (shaded in dark grey), and the length of the 5' and 3' overhangs of the antisense strand over the sense strand; and portrays the exemplary PD-L1 5'-terminal (SEQ ID NO. 139) and 3' terminal (SEQ ID NO. 165) mRNA sequences.

[0039] Without being limited to any particular theory or analysis and with respect to the exemplified RNA molecules, FIG. 1B sorts the exemplary RNA molecules 1-46 according to %GC content, FIG. 1C aligns exemplary PD-L1 target nucleotide sequences (e.g. SEQ ID NOs. 140, 141, 144, 147, 150, 155, 156, 158, 161 and 164) with an exemplary full-length PD-L1 (SEQ ID NO. 166); FIG. 1D shows the exemplary full-length PD-L1 mRNA (SEQ ID NO. 166) subdivided into 7 overlapping sequences (SEQ ID NOs.: 167-173), each of which can be targeted by a subset of the exemplary RNA molecules.

[0040] FIG. 2 shows the relative PD-L1 mRNA levels in a MDA-MB-231 cell line treated with either an exemplary RNA molecule or an aiGFP control according to an embodiment of the present teachings.

[0041] FIG. 3 is a histogram of an exemplary flow cytometry analysis of PD-L1 expression in a MDA-MB-231 cell line treated with either an exemplary RNA molecule or aiGFP control according to an embodiment of the present teachings.

[0042] FIG. 4 shows the mean fluorescent intensities of an exemplary flow cytometry analysis of PD-L1 expression in a MDA-MB-231 cell line treated with either an exemplary RNA molecule or aiGFP control according to an embodiment of the present teachings. Asterisk (*) denotes significant differences regarding the control calculated using Dunnett's Multiple comparison test ($p=0.0004$).

[0043] FIG. 5 shows the relative tumor cell-specific T cell cytotoxicity following the treatment of tumor cells with either an exemplary RNA molecule or a PD-L1 antibody according to an embodiment of the present teachings.

[0044] FIG. 6 shows the relative serum stability of an exemplary modified RNA molecule compared to an unmodified RNA molecule according to an embodiment of the present teachings.

[0045] FIG. 7 shows the potency of an exemplary modified RNA molecule according to an embodiment of the present teachings.

[0046] FIG. 8 shows the relative tumor cell-specific T cell cytotoxicity following the treatment of the tumor cells with an exemplary modified RNA molecule according to an embodiment of the present teachings.

[0047] FIG. 9 shows an exemplary embodiment of IL-2 expression in PD-1⁺ Jurkat co-cultured with aiScramble or aiPD-L1 transfected MDA-MB-231 cells.

[0048] The features and advantages of the present teachings may be more readily understood by those of ordinary skill in the art upon reading the following detailed description. It is to be appreciated that certain features of the present teachings that are, for clarity reasons, described above and below in the context of separate embodiments, may also be combined to form a single embodiment and that various features of the present teachings that are, for brevity reasons, described in the context of a single embodiment, may also be combined so as to form sub-combinations thereof. Embodiments identified herein as exemplary or preferred are intended to be illustrative and not limiting.

[0049] The methods and techniques of the present teachings are generally performed according to conventional methods well known in the art and as described in certain general and more specific references that are cited and discussed throughout the present teachings unless otherwise indicated. See, e.g., M.R. Green and J. Sambrook, *Molecular Cloning: A Laboratory Manual*, 4th ed., Cold

Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012), Ausubel *et al.*, Current Protocols, John Wiley & Sons, Inc. (2000-2016), Antibodies: A Laboratory Manual, 2nd edition, edited by Edward A. Greenfield, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2014), and RNA: A Laboratory Manual by Rio *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2011), all of which are incorporated herein by reference.

[0050] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In case of conflict, the present teachings, including definitions, will control. Methods and materials are described herein for use in the present teachings; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting.

[0051] As used herein, PD-L1 refers to a ligand of PD-1, also referred to in the art as CD274 molecule, CD274 antigen, B7 homolog, Programmed Cell Death 1 Ligand 1, PDCD1 ligand, PDCD1LG1, PDCD1L1, PD-L1, B7H1, PDL1, Programmed Death Ligand 1, B7-H1 or B7-H. The PD-L1 gene encodes an immune inhibitory receptor ligand that is expressed by hematopoietic and non-hematopoietic cells, such as T cells and B cells and various types of tumor cells. The encoded protein is a type I transmembrane protein that has immunoglobulin V-like and C-like domains. Interaction of this ligand with its receptor inhibits T-cell activation and cytokine production. During infection or inflammation of normal tissue, this interaction is important for preventing autoimmunity by maintaining homeostasis of the immune response. In tumor microenvironments, this interaction provides an immune escape for tumor cells through cytotoxic T-cell inactivation. Expression of this gene in tumor cells is considered to be prognostic in many types of human malignancies, including colon cancer and renal cell carcinoma. Alternative splicing results in at least 4 transcript variants. Other diseases associated with CD274 include, for example, lymphoepithelioma-like carcinoma and Paget's disease.

[0052] In certain embodiments, a PD-L1 expressed nucleotide sequence refers to a nucleotide sequence comprising at least 25 nucleotides of Homo sapiens CD274 molecule (CD274), transcript variant 1 (3,691 bp linear mRNA; NCBI Reference Sequence: NM_014143.3) having the sequence of SEQ ID No.: 166. Transcript variant 1. This variant represents the longest transcript and encodes the longest isoform (a).

[0053] In certain embodiments, a PD-L1 expressed nucleotide sequence can refer to a nucleotide sequence comprising at least 25 nucleotides of Homo sapiens CD274 molecule (CD274), transcript variant 2 (3,349 bp linear mRNA; Accession: NM_001267706.1). This variant lacks an alternate in-frame exon in the 5' coding region, compared to variant 1 which results in a shorter protein (isoform b), compared to isoform a.

[0054] In certain embodiments, a PD-L1 expressed nucleotide sequence can refer to a nucleotide sequence comprising at least 25 nucleotides of Homo sapiens CD274 molecule (CD274), transcript variant 3 (3,518 bp linear transcribed-RNA; Accession: NR_052005.1).

[0055] In certain embodiments, a PD-L1 expressed nucleotide sequence can refer to a nucleotide sequence comprising at least 25 nucleotides of Homo sapiens CD274 molecule (CD274), transcript variant 4 (907 bp linear mRNA; Accession: NM_001314029.1).

[0056] Unless specifically stated otherwise, references made in the singular may also include the plural. For example, “a” and “an” may refer to either one or one or more.

[0057] The phrase “and/or,” as used herein in the present teachings and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[0058] When a range of values is listed herein, it is intended to encompass each value and sub-range within that range. For example, “1-5 nucleotides” is intended to encompass 1 nucleotide, 2 nucleotides, 3 nucleotides, 4 nucleotides, 5 nucleotides, 1-2 nucleotides, 1-3 nucleotides, 1-4 nucleotides, 1-5 nucleotides, 2-3 nucleotides, 2-4 nucleotides, 2-5 nucleotides, 3-4 nucleotides, 3-5 nucleotides, or 4-5 nucleotides.

[0059] When the term “about” is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below those numerical values. In general, the term “about” is used herein to modify a numerical value above and below the stated value by a variance of 20%, 10%, 5%, or 1%. In some embodiments, the term “about” is used to modify a numerical

value above and below the stated value by a variance of 10%. In certain embodiments, the term “about” is used to modify a numerical value above and below the stated value by a variance of 5%. In certain embodiments, the term “about” is used to modify a numerical value above and below the stated value by a variance of 1%.

[0060] The term “acceptable” refers to being compatible with the other ingredients of the formulation and not injurious to the patient.

[0061] The term “acyclic nucleotide” as used herein generally refers to any nucleotide having an acyclic ribose sugar, for example, where any of the ribose carbon/carbon or carbon/oxygen bonds are independently or in combination absent from the nucleotide.

[0062] The term “alkyl,” as used herein, generally refers to saturated or unsaturated hydrocarbons, including straight-chain, branched-chain alkyl, alkenyl, and alkynyl groups, and cyclic groups, excluding aromatic groups. Notwithstanding the foregoing, alkyl also refers to non-aromatic heterocyclic groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted, the substituted group(s) is preferably, hydroxyl, halogen, cyano, C1-C4 alkoxy, =O, =S, NO₂, SH, NH₂ or NR₁R₂, where R₁ and R₂ independently can be H or C1-C4 alkyl. Preferably, the alkyl group is a C1-C4 alkyl group.

[0063] The term “aryl,” as used herein, generally refers to an aromatic group that has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which can be optionally substituted. The preferred substituent(s) of aryl groups can be halogen, trihalomethyl, hydroxyl, SH, OH, cyano, C1-C4 alkoxy, C1-C4 alkyl, C2-C4 alkenyl, C2-C4 alkynyl, NH₂ and NR₁R₂, where R₁ and R₂ independently can be H or C1-C4 alkyl.

[0064] The term “alkylaryl” as used herein generally refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups can be groups wherein the ring atoms on the aromatic ring can be all carbon atoms. The carbon atoms can be optionally substituted. Heterocyclic aryl groups can be groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms can be carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and examples of heterocyclic aryl

groups having such heteroatoms include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted.

[0065] The term “amide” as used herein generally refers to an —C(O)—NH—R , where R can be either alkyl, aryl, alkylaryl, or hydrogen.

[0066] The term “antisense region” as used herein refers to what is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the term refers to a nucleotide sequence of an aiRNA molecule having complementarity to an expressed nucleic acid sequence of a target gene. In addition, the antisense region of an aiRNA molecule can optionally comprise a nucleic acid sequence having complementarity to a sense region of the aiRNA molecule. In certain embodiments, the antisense region of the aiRNA molecule is referred to as the antisense strand or guide strand. In certain embodiments, the antisense strand can have 14, 15, 16 or 17 nucleotides that base pair with the sense strand. In certain embodiments, the antisense strand can have 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 nucleotides that are not contiguous with the corresponding colinear complementary nucleotides in a target nucleotide sequence. In certain embodiments, the antisense strand can have 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 nucleotides that are not complementary to the corresponding colinear nucleotides in a target nucleotide sequence. In certain embodiments, 19, 20 or 21 nucleotides of the antisense strand can be colinear with the corresponding complementary nucleotides in a target nucleotide sequence.

[0067] The terms “asymmetric interfering nucleic acid”, “aiRNA”, “asymmetric interfering oligonucleotide molecule”, or “chemically modified asymmetric interfering nucleic acid molecule” refer to any nucleic acid molecule that has an antisense strand and a sense strand, and the lengths of the two strands can be different. These nucleic acid molecules can inhibit or down-regulate gene expression or viral replication by mediating RNA interference (“RNAi”) or gene silencing in a sequence-specific manner. These terms can refer to both individual nucleic acid molecules, a plurality of such nucleic acid molecules, or pools of such nucleic acid molecules. The aiRNA can be a double-stranded nucleic acid molecule comprising complementary sense and antisense strands, wherein the antisense strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises a nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The aiRNA can also be a double-stranded nucleic acid molecule comprising

complementary sense and antisense strands, wherein the antisense strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises a nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof and has a nick (missing one nucleotide) or a gap (missing two or more nucleotides).

[0068] The term “biological system” as used herein generally refers to material, in a purified or unpurified form, from biological sources including, but not limited to, human or animal, wherein the system comprises the components required for RNAi activity. Thus, the term includes, for example, a cell, tissue, subject, or organism, or extract thereof. The term also includes reconstituted material from a biological source.

[0069] The term “blunt end” as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the term refers to a terminus of a double-stranded aiRNA molecule having no overhanging nucleotides. For example, the two strands of a double-stranded aiRNA molecule having blunt ends align with each other with matched base-pairs without overhanging nucleotides at the termini. An aiRNA duplex molecule of the present teachings can comprise blunt ends at one or both termini of the duplex, such as the terminus located at the 5'-end of the antisense strand, the 5'-end of the sense strand, or both termini of the duplex. In certain embodiments, a blunt end is formed when the 3'-terminal nucleotide of the sense strand base pairs with the 5'-terminal nucleotide of the antisense strand.

[0070] The term “cell” as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the term can be used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human being. The cell can be present in an organism, e.g., birds, plants, and mammals, such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell). The cell can be of somatic or germ line organ, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

[0071] The term “chemical modification” as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the term refers to any modification of the chemical structure of the nucleotides that differs from

nucleotides of a native nucleic acid in general. The term “chemical modification” encompasses, for example, the addition, substitution, or modification of native RNA at the sugar, base, or internucleotide linkage, as described herein or as is otherwise known in the art. In certain embodiments, the term “chemical modification” can refer to certain forms of RNA that are naturally occurring in certain biological systems, for example 2'-O-methyl modifications or inosine modifications.

[0072] The term “complementarity” or “complementary” as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the terms generally refer to the formation or existence of hydrogen bond(s) between one nucleic acid sequence and another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types of bonding as described herein, forming a base-paired, double-stranded region. In reference to the nucleic molecules of the present teachings, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. “Perfect complementarity” means that all the contiguous residues of a nucleic acid sequence can hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. “Partial complementarity” can include various mismatches or non-base paired nucleotides (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more mismatches, non-nucleotide linkers, or non-base paired nucleotides) within the nucleic acid molecule, which can result in bulges, loops, or overhangs between the sense strand or sense region and the antisense strand or antisense region of the nucleic acid molecule or between the antisense strand or antisense region of the nucleic acid molecule and a corresponding target nucleic acid molecule. Such partial complementarity can be represented by a % complementarity that is determined by the number of non-base paired nucleotides, i.e., about 20%-99% depending on the total number of nucleotides involved. Such partial complementarity is permitted to the extent that the nucleic acid molecule (e.g. aiRNA) maintains its function, for example the ability to mediate sequence specific RNAi. “Substantial complementarity” means that the sequences are sufficiently complementary to each other to hybridize under selected reaction conditions. Two substantially complementary strands can be, for example, perfectly complementary or can contain from 1 to many mismatches so long as the hybridization conditions are sufficient to allow, for example, discrimination between a pairing sequence and a non-pairing sequence. Accordingly, substantially complementary sequences can refer to sequences with base-pair complementarity of about 50%-

100% in a double-stranded region. The term “complementarity” in certain embodiments can refer to “perfect complementarity,” “partial complementarity,” or “substantial complementarity.”

[0073] A ribonucleotide consists of a phosphate group, a ribose sugar group, and a nucleobase that can be either adenine (A), guanine (G), cytosine (C), or uracil (U). An RNA strand refers to a chain of ribonucleotides linked together by phosphodiester bonds between the 5'-phosphate of one nucleotide and the 3' hydroxyl group of the next nucleotide. However, in certain embodiments, the chain of ribonucleotides may comprise bonds other than phosphodiester bonds between the 5'-phosphate of one nucleotide and the 3' hydroxyl group of the next nucleotide.

[0074] In double-stranded or duplex RNAs, one or more ribonucleotides of one strand stably associates with a complementary ribonucleotide in the other strand. The complementarity between the strands is brought about by the interaction or "base pairing" between A and U, and between G and C (see, for example, TABLE IA).

TABLE IA

C	C	U	A	U	A	U	G	U	G	G	U	A	G	A	SEQ ID NO. 174
•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
G	G	A	U	A	U	A	C	A	C	C	A	U	C	U	SEQ ID NO. 175

TABLE IB

C	C	U	U	U	A	U	U	U	G	G	U	A	G	A	SEQ ID NO. 176
•	•	•		•	•	•		•	•	•	•	•	•	•	
G	G	A	U	A	U	A	C	A	C	C	A	U	C	U	SEQ ID NO. 175

[0075] In certain embodiments, an RNA duplex can have RNA strands that are either perfectly complementary or partially complementary, depending on the number of mismatched, i.e., non-base paired nucleotides present in the RNA duplex (see, for example, TABLE IB).

[0076] As used herein, the term “align” refers to the process of comparing the nucleotide sequence of two or more nucleotide sequences to assess their degree of sequence identity. As used herein, a “match” refers to the alignment of two or more nucleotide sequences having 100% sequence identity.

[0077] Thus, for example, in TABLE II below, ROW 1 is aligned and matches ROW 2.

TABLE II

ROW 1	C	C	U	A	U	A	U	G	U	G	G	U	A	G	A	SEQ ID NO. 174
ROW 2	C	C	U	A	U	A	U	G	U	G	G	U	A	G	A	SEQ ID NO. 174
	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
ROW 3	G	G	A	U	A	U	A	C	A	C	C	A	U	C	U	SEQ ID NO. 175

[0078] Because the identity of a base on one strand of a RNA duplex can be used to infer the identity of the corresponding base on the other strand, the term "align" can also refer to the comparison between the nucleotide sequence of one strand (sense strand) and its complementary sequence in another RNA strand (antisense strand). Thus, for example, in TABLE II, ROW 2 is aligned with ROW 3 because the complementary sequence of the nucleotide sequence of ROW 3 matches the nucleotide sequence in ROW 2.

[0079] For example, in certain embodiments, the nucleotide sequence of an aiRNA's antisense strand can be aligned with its perfectly complementary or partially complementary nucleotide sequence in a target nucleotide sequence.

[0080] Generally, the term "contiguous" refers to those nucleotides that are immediately adjacent to each other in a polynucleotide chain.

[0081] In certain embodiments, the term "contiguous" can refer to those nucleotides that are adjacent to each other in a polynucleotide chain that match the corresponding nucleotides in a second polynucleotide chain.

[0082] Thus, for example in TABLE III, the nucleotides from position 1 to 11 of RNA strand 2 are contiguous with the corresponding nucleotides 1 to 11 of RNA strand 1 because nucleotides 1-11 of RNA strand 2 match the nucleotides 1-11 of RNA strand 1 without any intervening mismatches.

[0083] In certain embodiments, the term "contiguous" can also refer to those nucleotides that are adjacent to each other in a first polynucleotide chain that align with perfectly complementary nucleotides in a second polynucleotide chain.

[0084] Thus, for example in TABLE III, the nucleotides of RNA strand 1 are contiguous with the nucleotides in RNA strand 3 because each nucleotide of RNA strand 1 aligns with the corresponding complimentary nucleotide in RNA strand 3 without any intervening mismatches.

TABLE III

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
RNA STRAND 1 (SEQ ID NO. 174)	C	C	U	A	U	A	U	G	U	G	G	U	A	G	A	
RNA STRAND 2 (SEQ ID NO. 177)	C	C	U	A	U	A	U	G	U	G	G	G	G	G	A	
	●	●	●	●	●	●	●	●	●	●	●			●	●	
RNA STRAND 3 (SEQ ID NO. 175)	G	G	A	U	A	U	A	C	A	C	C	A	U	C	U	
RNA strand 4 (SEQ ID NO. 178)	G	G	A	A	U	A	U	A	C	A	C	C	A	U	C	U

[0085] Generally, the term "colinear" describes the 1:1 relationship between the linear order of nucleotides in a first RNA strand and the linear order of nucleotides in a second RNA strand.

[0086] Thus, for example in TABLE III, RNA strand 2 is colinear with RNA strand 1 despite the lack of sequence identity at positions 12 and 13 because the linear order of nucleotides 1-11, 14 and 15 of RNA strand 2 matches the corresponding nucleotides of RNA strand 1.

[0087] In certain embodiments, the term "colinear" also describes the 1:1 relationship between the linear order of nucleotides in a first RNA strand and the linear order of the corresponding complementary nucleotides in a second RNA strand.

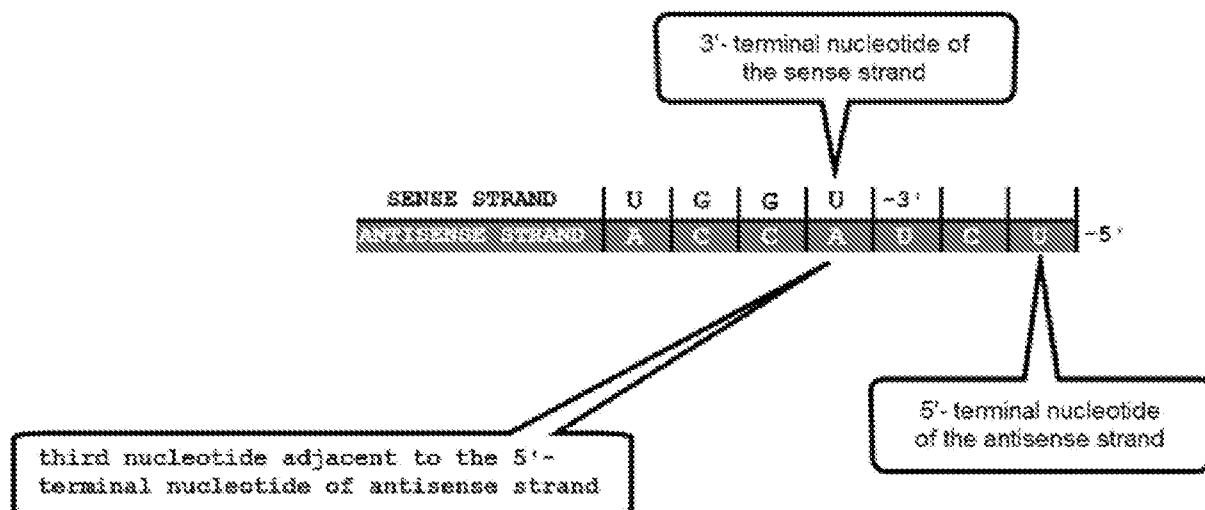
[0088] Thus, for example in TABLE III, RNA strand 2 is colinear with RNA strand 3 despite the partial complimentary between the two strands because the linear order of nucleotides 1-11, 14 and 15 of RNA strand 2 aligns with the corresponding complementary nucleotides at positions 1-11, 14 and 15 of RNA strand 3.

[0089] In contrast, RNA strand 3 is not colinear with RNA strand 4 because the linear order of nucleotides in RNA strand 3 does not match the linear order of nucleotides in RNA strand 4 as a result of the insertion of an extra nucleotide at position 3. Similarly, the linear order of the nucleotides in RNA strand 2 is not colinear with the linear order of the corresponding complementary nucleotides in RNA strand 4 again because of the extra nucleotide at position 3.

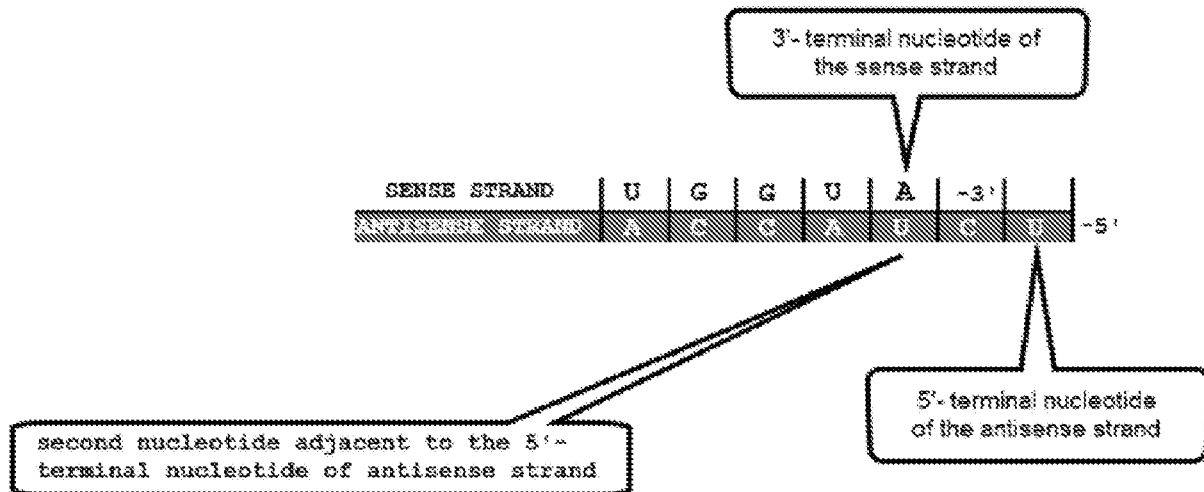
[0090] In certain embodiments, the term "colinear" refers to the 1:1 relationship between the linear order of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 or more nucleotides in an aiRNA's antisense strand and the linear order of the corresponding complementary nucleotides in a target nucleotide sequence. In certain embodiments, the aiRNA's antisense strand may have either perfect or partial complementarity with the sense strand.

[0091] In certain embodiments, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 or more nucleotides in the aiRNA's antisense strand are contiguous with the corresponding complementary nucleotides in a target nucleotide sequence. In certain embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 or more nucleotides in the aiRNA's antisense strand are not contiguous with the corresponding complementary nucleotides in a target nucleotide sequence.

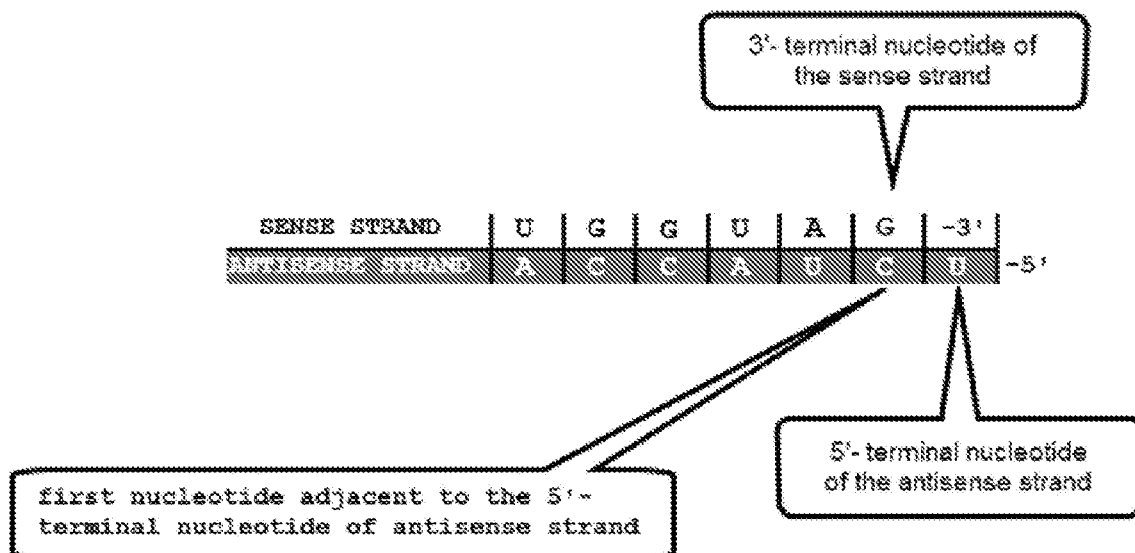
[0092] An exemplary 5' overhang of an aiRNA of the present teachings in which the 3'-terminal nucleotide of the sense strand is complementary to the third nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand is shown below:



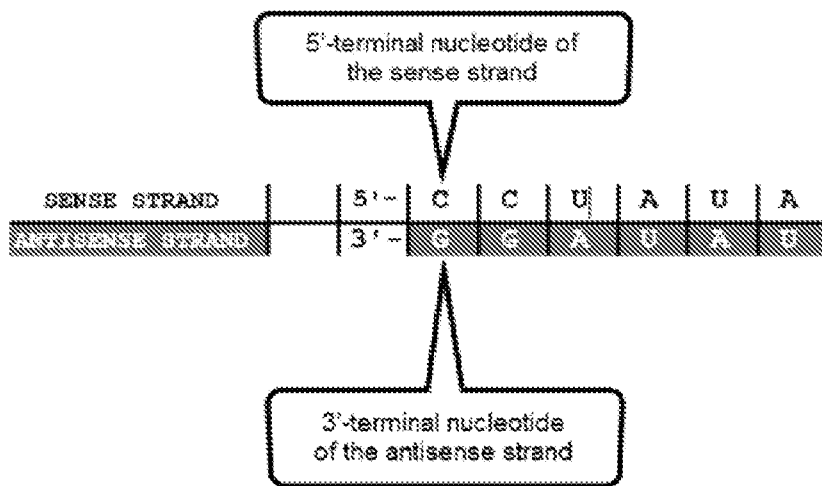
[0093] An exemplary 5' overhang of an aiRNA of the present teachings in which the 3'-terminal nucleotide of the sense strand is complementary to the second nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand is shown below:



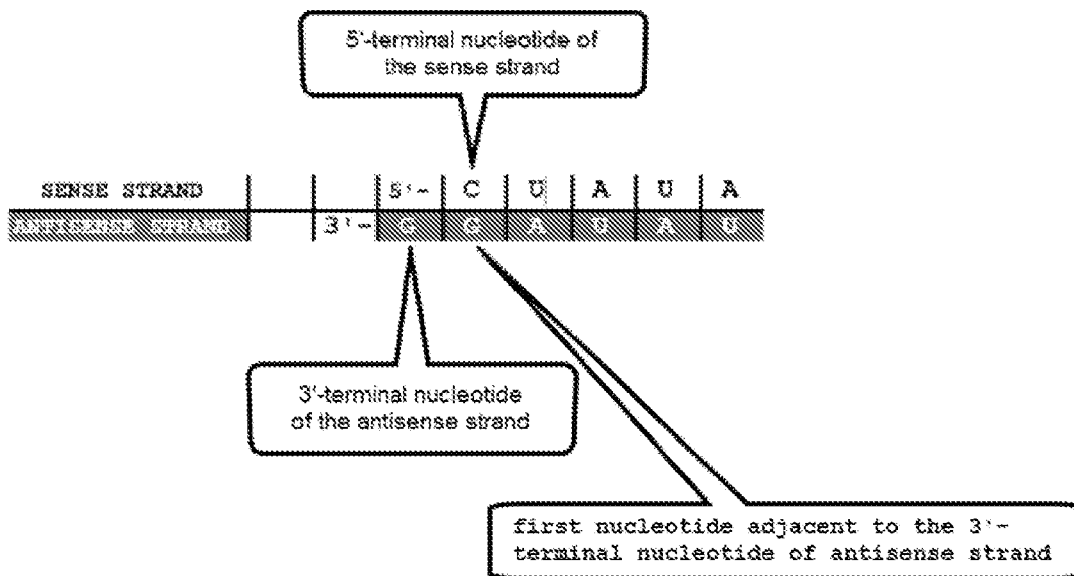
[0093] An exemplary 5' overhang of an aiRNA of the present teachings in which the 3'-terminal nucleotide of the sense strand is complementary to the first nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand is shown below:



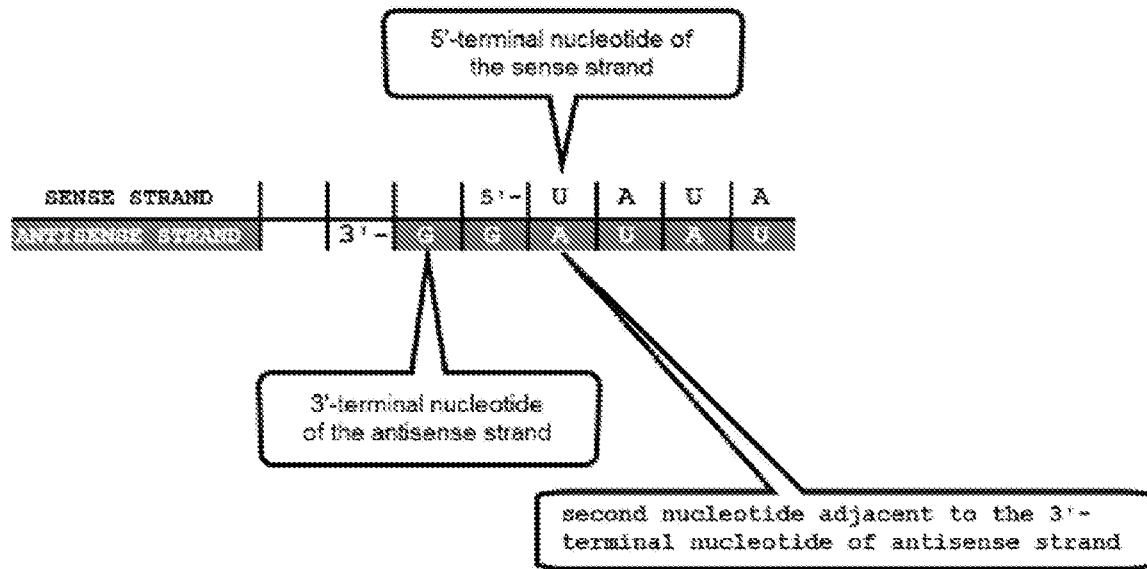
[0094] An exemplary depiction of an aiRNA of the present teachings in which the 5'-terminal nucleotide of the sense strand is complementary to the 3'-terminal nucleotide of the antisense strand is shown below:



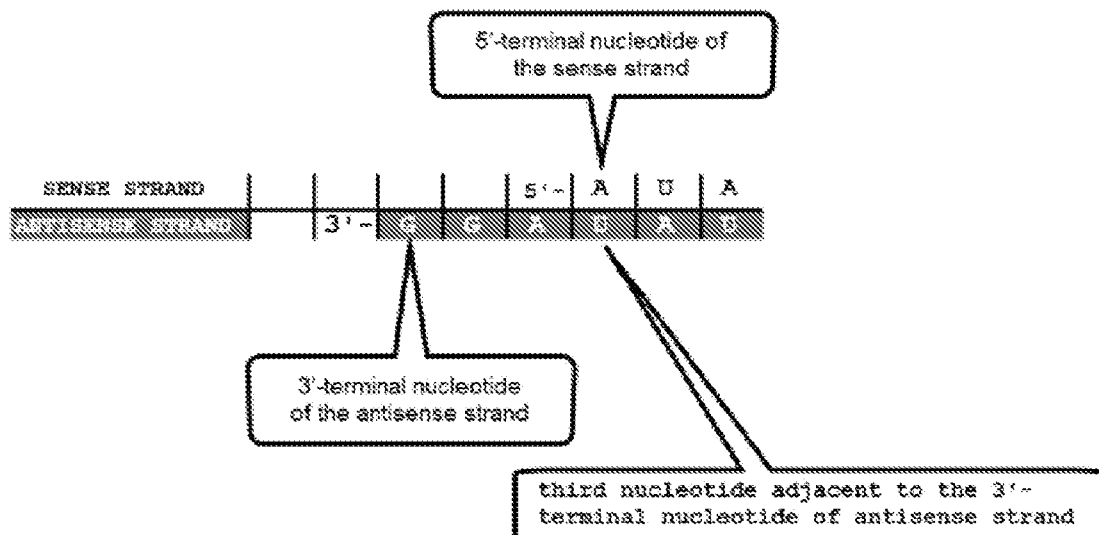
[0095] An exemplary 3' overhang of an aiRNA of the present teachings in which the 5'-terminal nucleotide of the sense strand is complementary to the first nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand is shown below:



[0096] An exemplary 3' overhang of an aiRNA of the present teachings in which the 5'-terminal nucleotide of the sense strand is complementary to the second nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand is shown below:



[0097] An exemplary 3' overhang of an aiRNA of the present teachings in which the 5'-terminal nucleotide of the sense strand is complementary to the third nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand is shown below:



[0098] The terms “composition” or “formulation” as used herein refer to their generally accepted meaning in the art. These terms generally refer to a composition or formulation, such as in a pharmaceutically acceptable carrier or diluent, in a form suitable for administration, e.g., systemic or local administration, into a cell or subject, including, for example, a human. Suitable forms, in part, depend upon the use or the route of entry, for example, oral, transdermal, inhalation, or by injection. Such forms should not prevent the composition or formulation from reaching a

target cell (i.e., a cell to which the negatively charged nucleic acid is desirable for delivery). For example, compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect. As used herein, pharmaceutical formulations include formulations for human and veterinary use. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the present teachings include: lipid nanoparticles (see for example Semple et al., 2010, *Nat Biotechnol.*, February; 28 (2):172-6); P-glycoprotein inhibitors (such as Pluronic P85); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery (Emerich, D F et al, 1999, *Cell Transplant*, 8, 47-58); and loaded nanoparticles, such as those made of polybutylcyanoacrylate. Other non-limiting examples of delivery strategies for the nucleic acid molecules of the present teachings include material described in Boado et al., 1998, *J. Pharm Sci.*, 87, 1308-1315; Tyler et al., 1999, *FEBS Lett.*, 421, 280-284; Pardridge et al., 1995, *PNAS USA.*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.*, 15, 73-107; Aldrian-Herrada et al., 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler et al., 1999, *PNAS USA.*, 96, 7053-7058.

[0099] As used herein, the term "cancer" in a subject refers to cells having uncontrolled proliferation, immortality, metastatic potential, rapid growth and increased proliferation rate, as well as certain morphological features. Cancer cells can aggregate in the form of a tumors or masses and/or circulate in the blood stream or lymphatic system as independent cells.

[0100] The term "cancer" comprises, for example, AIDS-Related cancers, breast cancers, cancers of the digestive/gastrointestinal tract, endocrine and neuroendocrine cancers, cancers of the eye, genitourinary cancers, germ cell cancers, gynecologic cancers, head and neck cancers, hematologic cancers, musculoskeletal cancers, neurologic cancers, respiratory/thoracic cancers, skin cancers, childhood cancers as well as cancers of unknown primary.

[0101] Exemplary AIDS-related cancers include, but are not limited to, AIDS-Related Lymphoma, Primary Central Nervous System Lymphoma and Kaposi Sarcoma.

[0102] Exemplary breast cancers include, but are not limited to, ductal carcinomas in situ (DCIS), invasive ductal carcinomas (IDC), invasive lobular carcinoma (ILC), triple negative breast cancers (where the tumor cells are negative for progesterone, estrogen, and her2/neu receptors), inflammatory breast cancers, metastatic breast cancers, breast cancers during pregnancy, Paget

disease of the nipple, Phyllodes tumor, adenoid cystic (or adenocystic) carcinoma, low-grade adenosquamous carcinoma, medullary carcinomas, tubular carcinomas, papillary carcinoma, mucinous (colloid) carcinomas, lymphoma of the breast, adenomyoepithelioma, giant cell sarcoma of the breast, leiomyosarcoma of the breast, angiosarcoma of the breast, cystosarcoma phylloides, and liposarcoma of the breast, carcinoid tumors of the breast, acinic cell carcinoma, oncocytic carcinoma (mammary epithelial oncocytoma), mucoepidermoid carcinoma, spindle cell carcinoma of the breast, squamous cell carcinoma of the breast, secretory carcinoma of the breast (juvenile secretory carcinoma), metaplastic carcinoma of the breast, invasive micropapillary carcinoma of the breast, adenoid cystic carcinoma of the breast, cribriform carcinoma, myofibroblastoma of the breast (benign spindle stromal tumor of the breast) and glycogen-rich clear cell carcinoma of the breast.

[0103] Exemplary cancers of the digestive/gastrointestinal tract include, but are not limited to, anal cancer, appendix cancer, gastrointestinal carcinoid tumor, bile duct cancer, carcinoid tumor, gastrointestinal cancer, colon cancer, esophageal cancer, gallbladder cancer, gastrointestinal stromal tumors (GIST), islet cell tumors, pancreatic neuroendocrine tumors, liver cancer, pancreatic cancer, rectal cancer, small intestine cancer, gastro-esophageal junction (GEJ) cancer, and stomach (gastric) cancer.

[0104] Exemplary endocrine and neuroendocrine cancers include, but are not limited to, adrenocortical carcinomas, gastrointestinal carcinoid tumors, islet cell tumors, pancreatic neuroendocrine tumors, Merkel cell carcinomas, non-small cell lung neuroendocrine tumors, small cell lung neuroendocrine tumors, parathyroid cancers, pheochromocytomas, pituitary tumors, and thyroid cancers.

[0105] Exemplary genitourinary cancers include, but are not limited to, bladder cancer, kidney (renal cell) cancer, penile cancer, prostate cancer, renal pelvis and ureter cancer, transitional cell, testicular cancer, urethral cancer, Wilms tumor and other childhood kidney tumors.

[0106] Exemplary gynecologic cancers include, but are not limited to, cervical cancer, endometrial cancer, fallopian tube cancer, gestational trophoblastic tumor, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, primary peritoneal cancer, uterine sarcoma, vaginal cancer and vulvar cancer.

[0107] Exemplary head and neck cancers include, but are not limited to, hypopharyngeal cancer, laryngeal cancer, lip and oral cavity cancer, metastatic squamous neck cancer with occult primary, mouth cancer, nasopharyngeal cancer, oral cavity cancer, lip and oropharyngeal cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, pharyngeal cancer, salivary gland cancer, throat cancer and thyroid cancer.

[0108] Exemplary hematologic cancers include, but are not limited to, leukemias, acute lymphoblastic leukemia, adult, childhood acute lymphoblastic leukemia, adult acute myeloid leukemia, childhood acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia, lymphomas, AIDS-related lymphoma, cutaneous T-cell lymphoma, adult Hodgkin lymphoma, childhood Hodgkin lymphoma, Hodgkin lymphoma during pregnancy, mycosis fungoides, childhood Non-Hodgkin lymphoma, adult Non-Hodgkin lymphoma, Non-Hodgkin lymphoma during pregnancy, primary central nervous system lymphoma, Sézary syndrome, cutaneous T-cell lymphoma, Waldenström macroglobulinaemia, chronic myeloproliferative neoplasms, Langerhans cell histiocytosis, multiple myeloma/plasma cell neoplasm, myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms.

[0109] Exemplary musculoskeletal cancers include, but are not limited to, bone cancer, Ewing's sarcoma, osteosarcoma, malignant fibrous histiocytoma of bone, childhood rhabdomyosarcoma and soft tissue sarcoma.

[0110] Exemplary neurologic cancers include, but are not limited to, adult brain tumor, childhood brain tumor, astrocytomas, brain and spinal cord tumors, brain stem glioma, atypical teratoid/rhabdoid central nervous system tumor, embryonal central nervous system tumors, germ cell central nervous system tumors, craniopharyngioma, ependymoma, neuroblastoma, pituitary tumor and primary central nervous system (CNS) lymphoma

[0111] Exemplary respiratory/thoracic cancers include, but are not limited to, non-small cell lung cancer, small cell lung cancer, malignant mesothelioma, thymoma and thymic carcinoma.

[0112] Exemplary skin cancers include, but are not limited to, cutaneous T-cell lymphoma, Kaposi sarcoma, melanoma, Merkel cell carcinoma, skin cancer, cutaneous T-cell lymphoma, mycosis fungoides and Sézary syndrome.

[0113] Also included within the term “cancer” is “solid tumor.” As used herein, the term “solid tumor” refers to those conditions, such as cancer, that form an abnormal tumor mass, such as sarcomas, carcinomas, and lymphomas. Examples of solid tumors include, but are not limited to, non-small cell lung cancer (NSCLC), neuroendocrine tumors, thyomas, fibrous tumors, metastatic colorectal cancer (mCRC), and the like. In certain embodiments, the solid tumor disease can be an adenocarcinoma, squamous cell carcinoma, large cell carcinoma, and the like.

[0114] With reference to exemplary nucleic acid molecules of the present teachings, the term “correspond” or “correspondence” as used herein refers to the relationship between a target nucleotide sequence and a sequence in the antisense strand of an aiRNA molecule of the present teachings. The term can be modified by the word “partially,” “substantially,” or “completely” to indicate the degree of the relationship. In certain embodiments, a partial correspondence means about 20-99% of the ribonucleotides A, U, G, and C in the antisense strand of aiRNA sequence is complementary to its corresponding target nucleotide sequence. In certain embodiments, a substantial correspondence means about 50%-100% of the ribonucleotides A, U, G, and C in the antisense strand of aiRNA sequence are complementary to the corresponding target nucleotide sequence. In certain embodiments, the target nucleotide sequence can be a PD-L1 mRNA sequence. In certain embodiments, the target nucleotide sequence can be the nucleotide sequence of SEQ ID NO. 166. In certain embodiments, the target nucleotide sequence comprises a sequence chosen from SEQ ID NOs. 93-138.

[0115] The term “cytotoxic/cytostatic agents” refer to compounds that cause cell death or inhibit cell proliferation primarily by interfering directly with the cell’s functioning or inhibit or interfere with cell mitosis, including alkylating agents, tumor necrosis factors, intercalators, hypoxia activatable compounds, microtubule inhibitors/microtubule-stabilizing agents, inhibitors of mitotic kinesins, inhibitors of histone deacetylase, inhibitors of kinases involved in mitotic progression, antimetabolites; biological response modifiers; hormonal/anti-hormonal therapeutic agents, hematopoietic growth factors, monoclonal antibody targeted therapeutic agents, topoisomerase inhibitors, proteasome inhibitors and ubiquitin ligase inhibitors.

[0116] The term “deoxyribonucleotide” as used herein refers to its meaning as is generally accepted in the art. The term generally refers to a nucleotide with a proton at the 2’ position of a

β -D-deoxyribofuranose moiety. This term also includes any deoxyribonucleotides that are chemically modified. "dT" refers to 2'-deoxythymidine.

[0117] As used herein, a "polynucleotide" refers to a polymeric chain containing two or more nucleotides. "Polynucleotides" includes primers, oligonucleotides, nucleic acid strands, etc. A polynucleotide may contain standard or non-standard nucleotides. Typically, a polynucleotide contains a 5' phosphate at one terminus ("5' terminus") and a 3' hydroxyl group at the other terminus ("3' terminus") of the chain. The most 5' nucleotide of a polynucleotide may be referred to herein as the "5'-terminal nucleotide" of the polynucleotide. The most 3' nucleotide of a polynucleotide may be referred to herein as the "3'-terminal nucleotide" of the polynucleotide.

[0118] As used herein, a "double stranded RNA," a "duplex RNA" or a "RNA duplex" refers to an RNA of two strands and with at least one double-stranded region, and includes RNA molecules that have, for example, at least one gap, nick, bulge, and/or bubble either within a double-stranded region or between two neighboring double-stranded regions. In certain embodiments, if one strand has a gap or a single-stranded region of unmatched nucleotides between two double-stranded regions, that strand is considered as having multiple fragments. In certain embodiments, a double-stranded RNA as used here can have terminal overhangs on either end or both ends. In certain embodiments, the two strands of the duplex RNA can be linked through a chemical linker.

[0119] As used herein, the term "effective amount" of an active agent refers to an amount sufficient to elicit the desired biological response. As will be appreciated by those of ordinary skill in this art, the effective amount of an RNA molecule of the present teachings may vary depending on such factors as the desired biological endpoint, the pharmacokinetics of the molecule, the disease being treated, the mode of administration, and the patient.

[0120] An "effective amount" of an anti-cancer agent in reference to decreasing cancer cell growth means an amount capable of decreasing, to some extent, the growth and/or metastasis of some cancer or tumor cells. The term includes an amount capable of invoking a growth inhibitory, cytostatic and/or cytotoxic effect, and/or apoptosis of the cancer or tumor cells.

[0121] The term "gene" or "target gene" or "target nucleotide sequence" as used herein refers to their meaning as is generally accepted in the art. The terms generally refer a target nucleotide (e.g., DNA or RNA) sequence that comprises partial length or entire length coding sequences

necessary for the production of a polypeptide, e.g. PD-L1 or PD-L2. In certain embodiments, the target nucleotide sequence can also include the untranslated region (UTR) or non-coding region of the target gene. In certain embodiments, a gene or target gene can also encode a functional RNA (fRNA) or non-coding RNA (ncRNA), such as small temporal RNA (stRNA), micro RNA (miRNA), small nuclear RNA (snRNA), short interfering RNA (siRNA), small nucleolar RNA (snoRNA), ribosomal RNA (rRNA), transfer RNA (tRNA) and precursor RNAs thereof. In certain embodiments, such non-coding RNAs can serve as target nucleotide sequences for aiRNA mediated RNA interference in modulating the activity of fRNA or ncRNA involved in functional or regulatory cellular processes. Aberrant fRNA or ncRNA activity leading to disease can therefore be modulated by aiRNA molecules of the present teachings. In certain embodiments, aiRNA molecules targeting fRNA and ncRNA can also be used to manipulate or alter the genotype or phenotype of a subject, organism or cell, by intervening in cellular processes such as genetic imprinting, transcription, translation, or nucleic acid processing (e.g., transamination, methylation etc.). In certain embodiments, the target gene can be a gene derived from a cell, an endogenous gene, a transgene, or exogenous genes such as genes of a pathogen, for example a virus, which is present in the cell after infection thereof. The cell containing the target gene can be derived from or contained in any organism, for example, a plant, animal, protozoan, virus, bacterium, or fungus. Non-limiting examples of plants include monocots, dicots, or gymnosperms. Non-limiting examples of animals include vertebrates or invertebrates. Non-limiting examples of fungi include molds or yeasts. For a review, see for example, Snyder and Gerstein, 2003, *Science*, 300, 258-260.

[0122] The term “homologous sequence” as used herein refers to its meaning as is generally accepted in the art. The term generally refers a nucleotide sequence that is shared by one or more polynucleotide sequences, such as genes, gene transcripts and/or non-coding polynucleotides. For example, a homologous sequence can be a nucleotide sequence that is shared by two or more genes encoding related but different proteins, such as different members of a gene family, different protein epitopes, different protein isoforms or completely divergent genes. A homologous sequence can be a nucleotide sequence that is shared by two or more non-coding polynucleotides, such as noncoding DNA or RNA, regulator sequences, introns, and sites of transcriptional control or regulation. Homologous sequences can also include sequence regions shared by more than one polynucleotide sequence. Homology does not need to be perfect identity (100%), as partially homologous sequences are also contemplated by and within the scope of the present teachings

(e.g., at least about 95%, about 94%, about 93%, about 92%, about 91%, about 90%, about 89%, about 88%, about 87%, about 86%, about 85%, about 84%, about 83%, about 82%, about 81%, about 80% etc.). Percent homology is the number of matching nucleotides between two sequences divided by the total length being compared, multiplied by 100.

[0123] The term “improved RNAi activity” refers to an increase in RNAi activity measured *in vitro* and/or *in vivo*, wherein the RNAi activity is a reflection of both the ability of the aiRNA to mediate RNAi and the stability of the aiRNAs of the present teachings. In the present teachings, the product of these activities can be increased *in vitro* and/or *in vivo* compared to a siRNA, a shRNA, or another RNA containing a plurality of ribonucleotides. In some cases, the activity or stability of the aiRNA molecule can be decreased (i.e., less than ten-fold), but the overall activity of the aiRNA molecule can be enhanced *in vitro* and/or *in vivo*.

[0124] The terms “inhibit,” “down-regulate,” or “reduce” as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the term generally refers the reduction in the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, below that observed in the absence of the nucleic acid molecules (e.g., aiRNA) of the present teachings. Down-regulation can also be associated with post-transcriptional silencing, such as, RNAi mediated cleavage or by alteration in DNA methylation patterns or DNA chromatin structure. Inhibition, down-regulation or reduction with an aiRNA molecule can be in reference to an inactive molecule, an attenuated molecule, an aiRNA molecule with a scrambled sequence, or an aiRNA molecule with mismatches or alternatively, it can be in reference to the system in the absence of the nucleic acid.

[0125] The terms “internucleoside linkage” or “internucleoside linker” or “internucleotide linkage” or “internucleotide linker” can be used herein interchangeably and refer to any linker or linkage between two nucleoside units, as is known in the art, including, for example, but not limited to, phosphate, analogs of phosphate, phosphonate, guanidium, hydroxylamine, hydroxythydrazinyl, amide, carbamate, alkyl, and substituted alkyl linkages. The internucleoside linkages constitute the backbone of a nucleic acid molecule.

[0126] The term “isolated” or “purified,” as used herein, refers to a material that is substantially or essentially free from components that normally accompany it in its native state.

Purity and homogeneity can be typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography.

[0127] The terms “mammalian” or “mammal” as used herein refers to its meaning as is generally accepted in the art. The term generally refers to any warm blooded vertebrate species, such as a human, mouse, rat, dog, cat, hamster, guinea pig, rabbit, livestock, and the like.

[0128] The term “modulate” as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the term refers to when the expression of a gene, or level of one or more RNA molecules (coding or non-coding), or activity of one or more RNA molecules or proteins or protein subunits, is up-regulated or down-regulated, such that the expression, level, or activity is greater than or less than that observed in the absence of the molecule that effects modulation. For example, the term “modulate” in certain embodiments can refer to inhibit/inhibition (e.g., gene silencing) and in other embodiments can refer to potentiate/potentialion or up-regulate/up-regulation, e.g., of gene expression. As used herein, “gene silencing” refers to reduction of gene expression, and may refer to a reduction of gene expression about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90% or about 95% of the targeted gene.

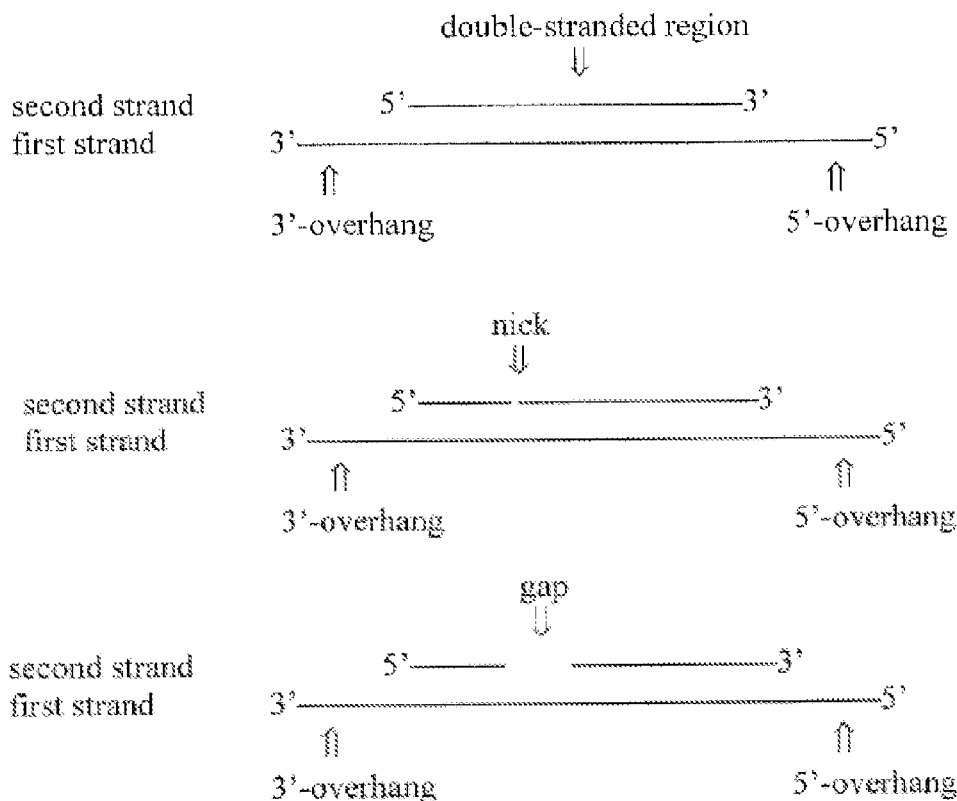
[0129] The term “modified nucleotide” as used herein refers to its meaning as is generally accepted in the art. The term generally refers a nucleotide, which contains a modification in the chemical structure of the base, sugar and/or phosphate of the unmodified (or natural) nucleotide as is generally known in the art.

[0130] The term “non-base paired” refers to nucleotides that are not base paired between the sense strand or sense region and the antisense strand or antisense region of a double-stranded aiRNA molecule; and can include , but is not limited to, for example, mismatches, overhangs, single stranded loops, etc.

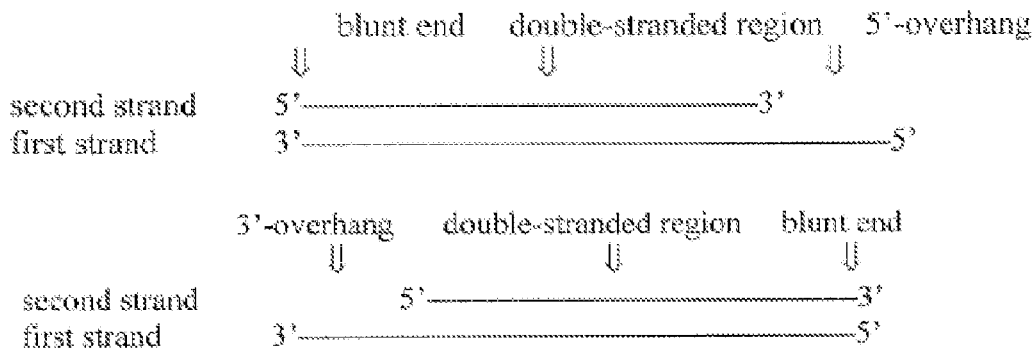
[0131] The term “nucleotide” (or “nt”) is used as is generally recognized in the art. Nucleotides generally comprise a nucleobase, a sugar, and an internucleoside linkage, e.g., a phosphodiester bond. The base can be a natural base (standard), modified bases, or a base analog. Such bases can be generally located at the 1'-position of a nucleotide sugar moiety. Additionally, the nucleotides can be unmodified or modified at the sugar, internucleoside linkage, and/or base moiety (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides,

non-standard nucleotides and others). In certain embodiments, a nucleotide can be a ribonucleotide (which sometimes refers to as a RNA nucleotide). In certain embodiments, a nucleotide can be a deoxyribonucleotide (which sometimes refers to as a DNA nucleotide).

[0132] The term “overhang” as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary double stranded nucleic acid molecules, the term generally refers to the terminal portion of a nucleotide sequence that is not base paired between the two strands of a double-stranded nucleic acid molecule. In certain embodiments, an overhang can be single stranded. In certain embodiments, the nucleic acid molecules of the present teachings include two overhangs at the antisense strand (i.e., 3'- and 5'-overhangs), as exemplified below.

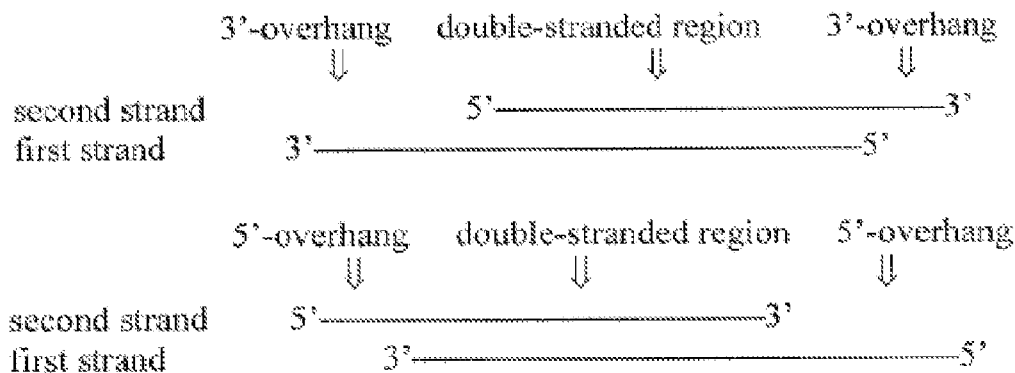


[0133] In certain embodiments, the nucleic acid molecules of the present teachings include one overhang and one blunt end (e.g., a 3'-overhang and a 5'-blunt end; or a 3'-blunt end and a 5'-overhang), as exemplified below.



[0134] In certain embodiments, the 3' overhang is not blunt.

[0135] Exemplary duplex RNAs having either two 3' overhangs or two 5' overhangs are depicted below.



[0136] The term “parenteral” as used herein refers to its meaning as is generally accepted in the art. The term generally refers to methods or techniques of administering a molecule, drug, agent, or compound in a manner other than through the digestive tract, and includes epicutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or intrathecal injection or infusion techniques and the like.

[0137] The terms “administer,” “administering,” or “administration” are used herein in their broadest sense. These terms refer to any method of delivering a compound or pharmaceutical composition described herein to a subject, cell or tumor by, for example, introducing a compound systemically, locally, or in situ to the subject. Thus, a compound of the present teachings produced in a subject from a composition (whether or not it includes the compound) is encompassed by these terms. When these terms are used in connection with the term “systemic” or “systemically,” they

generally refer to *in vivo* systemic absorption or accumulation of the compound or composition in the blood stream and its distribution throughout the entire body. In certain embodiments, the terms “administer,” “administering,” or “administration” can refer to, for example, delivering one or more recombinant vectors to a tumor cell, wherein the vector expresses an RNA interfering agent.

[0138] A “pharmaceutically acceptable composition” or “pharmaceutically acceptable formulation” can refer to a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the present teachings to the physical location most suitable for their desired activity.

[0139] The term “pharmaceutically acceptable excipient, carrier, or diluent” as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material. Some examples of materials which can serve as pharmaceutically acceptable excipients, carriers, or diluents include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate, magnesium stearate, and polyethylene oxide-polypropylene oxide copolymer as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[0140] The term “phosphorothioate” refers to an internucleotide phosphate linkage comprising one or more sulfur atoms in place of an oxygen atom. Hence, the term phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

[0141] The term “ribonucleotide” as used herein refers to its meaning as is generally accepted in the art. The term generally refers to a nucleotide with a hydroxyl group at the 2' position of a β -D-ribofuranose moiety. This term also includes any ribonucleotides that are chemically modified.

[0142] The term “RNA” as used herein refers to its generally accepted meaning in the art. Generally, the term RNA refers to a molecule comprising at least one ribofuranoside moiety. The term can include double-stranded RNA, single-stranded RNA, and isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the RNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in the RNA molecules of the present teachings can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

[0143] The term “RNA interference” or term “RNAi” refer to the biological process of inhibiting or down regulating gene expression in a cell, as is generally known in the art, and which can be mediated by short interfering nucleic acid molecules or asymmetric interfering nucleic acid molecules of the present teachings. Additionally, the term RNAi is meant to be equivalent to other terms used to describe sequence-specific RNA interference, such as post-transcriptional gene silencing, translational inhibition, transcriptional inhibition, or epigenetics. For example, aiRNA molecules of the present teachings can be used to epigenetically silence genes at either the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, modulation of gene expression by aiRNA molecules of the present teachings can result from aiRNA mediated cleavage of RNA (either coding or non-coding RNA) via RISC, or via translational inhibition, as is known in the art or modulation can result from transcriptional inhibition.

[0144] The term “sense region” as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the term refers to a nucleotide sequence of an aiRNA molecule having complementarity to an antisense region of the aiRNA molecule. In addition, the sense region of an aiRNA molecule can comprise a nucleic acid sequence having homology or sequence identity with a target nucleotide sequence. In one embodiment, the sense region of the aiRNA molecule is also referred to as the sense strand or passenger strand.

[0145] The terms “short interfering nucleic acid”, “siNA”, “short interfering RNA”, “siRNA”, “short interfering nucleic acid molecule”, “short interfering oligonucleotide molecule”, or “chemically modified short interfering nucleic acid molecule” refer to any nucleic acid molecule that is capable of inhibiting or down regulating gene expression or viral replication by mediating RNA interference (“RNAi”) or gene silencing in a sequence-specific manner and that includes an antisense strand and a sense strand, and the lengths of the two strands are the same. These terms can refer to both individual nucleic acid molecules, a plurality of such nucleic acid molecules, or pools of such nucleic acid molecules. The siRNA can be a double-stranded nucleic acid molecule comprising self-complementary sense and antisense strands, wherein the antisense strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises a nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. siRNAs can be symmetrical interfering RNAs having two 3' overhangs.

[0146] The term “subject” as used herein refers to its meaning as is generally accepted in the art. The term generally refers an organism to which the nucleic acid molecules of the present teachings can be administered. A subject can be a mammal or mammalian cell, including a human or human cell. The term also refers to an organism, which is a donor or recipient of explanted cells or the cells themselves. In certain embodiments, the term “subject” refers to any animal (e.g., a mammal), including, but not limited to humans, mammals and non-mammals, such as a non-human primate, a mouse, a rabbit, sheep, a dog, a cat, a horse, a cow, a chicken, an amphibian, a fish, an insect or a reptile which is to be the recipient of a particular treatment. Under some circumstances, the terms “subject” and “patient” can be used interchangeably herein in reference to a human subject.

[0147] The term “systemic administration” as used herein refers to its meaning as is generally accepted in the art. The term generally refers *in vivo* systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body.

[0148] The term “target site” or “target nucleotide sequence” as used herein refers to its meaning as is generally accepted in the art. The term generally refers to a sequence within a target nucleic acid molecule (e.g., RNA or DNA) that is “targeted.” With reference to an exemplary nucleic acid molecule of the present teachings, the target nucleotide sequence can be an expressed

nucleotide sequence, i.e. an RNA that is synthesized by an RNA polymerase that is targeted by an aiRNA. In certain embodiments, the target nucleotide sequence can be mRNA transcribed from a target gene. In certain embodiments, the antisense strand of an aiRNA can be colinear with the target nucleotide sequence. In certain embodiments, the antisense strand of an aiRNA can be perfectly complementary with at least 14 nucleotides of the target nucleotide sequence. In certain embodiments, the antisense strand of an aiRNA can be partially complementary to the target nucleotide sequence.

[0149] The term “therapeutically effective amount” as used herein refers to its meaning as is generally accepted in the art. The term generally refers to the amount of the compound or composition that will elicit the requisite biological or medical response in a cell, tissue, system, animal or human. For example, if a given clinical treatment is considered effective when there is at least about a 25% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is that amount necessary to effect at least about a 25% reduction in that parameter.

[0150] A “therapeutically effective amount” in reference to the treatment of cancer, means an amount capable of invoking one or more of the following effects: (1) inhibition, to some extent, of cancer or tumor growth, including slowing down growth or complete growth arrest; (2) reduction in the number of cancer or tumor cells; (3) reduction in tumor size; (4) inhibition (i.e., reduction, slowing down, or complete stopping) of cancer or tumor cell infiltration into peripheral organs; (5) inhibition (i.e., reduction, slowing down, or complete stopping) of metastasis; (6) enhancement of anti-tumor immune response, which may, but is not required to, result in the regression or rejection of the tumor, or (7) relief, to some extent, of one or more measurable symptoms associated with the cancer or tumor. The therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual and the ability of one or more anti-cancer agents to elicit a desired response in the individual. A “therapeutically effective amount” is also one in which any toxic or detrimental effects are outweighed by the therapeutically beneficial effects.

[0151] Terms such as “treating,” “treatment,” “to treat,” “alleviating,” or “to alleviate” as used herein refer to both (1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and (2) prophylactic or preventative

measures that prevent or slow the development of a targeted pathologic condition or disorder (“preventing” or “to prevent”). Thus those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented.

[0152] In certain embodiments, a subject is successfully “treated” according to the methods of the present teachings if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including the spread of cancer into soft tissue and bone; inhibition of or an absence of tumor metastasis; inhibition or an absence of tumor growth reduced morbidity and mortality. “Treatment” can also mean prolonging survival as compared to expected survival in the absence of treatment.

[0153] In certain embodiments, the term “treating cancer,” “treatment of cancer,” or an equivalent thereof, means to decrease, reduce, or inhibit the replication of cancer cells; decrease, reduce or inhibit the spread (formation of metastases) of cancer; decrease tumor size; decrease the number of tumors (i.e. reduce tumor burden); lessen or reduce the number of cancerous cells in the body; prevent recurrence of cancer after surgical removal or other anti-cancer therapies; or ameliorate measurable treatment endpoints (i.e., outcomes).

[0154] The term “up-regulate” as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the term refers to an increase in the expression of a gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more RNAs, proteins or protein subunits, above that observed in the absence of the nucleic acid molecules (e.g., aiRNA) of the present teachings. In certain embodiments, up-regulation or promotion of gene expression by an aiRNA molecule is above that level observed in the presence of an inactive or attenuated molecule. In certain embodiments, up-regulation or promotion of gene expression by an aiRNA molecule is above that level observed in the presence of, for example, an aiRNA molecule with scrambled sequence or with mismatches. In certain embodiments, up-regulation or promotion of gene expression by an aiRNA molecule is above that level observed in the presence of, for example, a siRNA molecule with the same, substantially the same, a similar antisense strand. In certain embodiments, up-regulation or promotion of gene expression by an aiRNA molecule is above that level observed in the presence of, for example, a biologically active molecule (e.g., a

small molecule, an antibody, or another biological) having a similar activity with respect to the expression of a target gene. In certain embodiments, up-regulation or promotion of gene expression with a nucleic acid molecule of the present teachings is greater in the presence of the nucleic acid molecule than in its absence. In certain embodiments, up-regulation or promotion of gene expression is associated with inhibition of RNA mediated gene silencing, such as RNAi mediated cleavage or silencing of a coding or non-coding RNA target that down regulates, inhibits, or silences the expression of a target gene.

[0155] The term “vector” as used herein refers to its meaning as is generally accepted in the art. The term vector generally refers to any nucleic acid- and/or viral-based expression system or technique used to deliver one or more nucleic acid molecules to a targeted cell or organism.

[0156] In one aspect, the present teachings provide an RNA molecule. In certain embodiments, an RNA molecule of the present invention includes a first strand and a second strand, where the second strand is complementary to the first strand and the first strand and the second strand form at least one double-stranded region. In certain embodiments, the first strand of the RNA molecule is longer than the second strand of the RNA molecule (length asymmetry). A RNA molecule with a length asymmetry of the present teachings is sometimes referred to as an aiRNA.

[0157] In certain embodiments, the first strand has a length of 5-100 nucleotides (nt.). In certain embodiments, the first strand has a length of 10-30 nt. In certain embodiments, the first strand has a length of 12-30 nt. In certain embodiments, the first strand has a length of 15-28 nt. In certain embodiments, the first strand has a length of 17-26 nt. In certain embodiments, the first strand has a length of 19-23 nt. In certain embodiments, the first strand has a length of 19 nt. In certain embodiments, the first strand has a length of 20 nt. In certain embodiments, the first strand has a length of 21 nt. In certain embodiments, the first strand has a length of 22 nt. In certain embodiments, the first strand has a length of 23 nt. In certain embodiments, the first strand is the antisense strand. In certain embodiments, the antisense strand can be 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 nucleotides long. In certain embodiments, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides of the antisense strand can be colinear with a PD-L1 mRNA sequence. In certain embodiments, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides of the antisense strand can be colinear and contiguous with a PD-L1

mRNA sequence. In certain embodiments, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 nucleotides of the antisense strand can be non-contiguous with a PD-L1 mRNA sequence.

[0158] In certain embodiments, the second strand has a length of 3-30 nt. In certain embodiments, the second strand has a length of 3-29 nt. In certain embodiments, the second strand has a length of 10-26 nt. In certain embodiments, the second strand has a length of 12-26 nt. In certain embodiments, the second strand has a length of 13-22 nt. In certain embodiments, the second strand has a length of 14-19 nt. In certain embodiments, the second strand has a length of 14-17 nt. In certain embodiments, the second strand has a length of 14 nt. In certain embodiments, the second strand has a length of 15 nt. In certain embodiments, the second strand has a length of 16 nt. In certain embodiments, the second strand has a length of 17 nt. In certain embodiments, the second strand has a length of 18 nt. In certain embodiments, the second strand has a length of 19 nt. In certain embodiments, the second strand is the sense strand. In certain embodiments, the sense strand can be 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 nucleotides long.

[0159] In certain embodiments, the first strand is at least 1 nt longer than the second strand. In certain embodiments, the first strand is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nt longer than the second strand. In certain embodiments, the first strand is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 nt longer than the second strand. In certain embodiments, the first strand is 2, 3, 4, 5, 6, 7, 8, 9, or 10 nt longer than the second strand. In certain embodiments, the first strand is 4, 5, 6, 7, 8, or 9 nt longer than the second strand.

[0160] In certain embodiments, the double-stranded region has a length of 3-98 base pairs (bp). In certain embodiments, the double-stranded region has a length of 3-28 bp. In certain embodiments, the double-stranded region has a length of 3-19 bp. In certain embodiments, the double-stranded region has a length of 14-19 bp. In certain embodiments where the aiRNA includes a two or more double-stranded region, each of the double-stranded regions has a length of 2-17 bp. In certain embodiments, the double-stranded region has a length of 14, 15, 16, 17, 18, or 19 bp. For example, the double-stranded region can have a length of 14, 15, 16, 17, 18, or 19 bp. In certain embodiments, the length of the double-stranded region can be 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 bp.

[0161] In certain embodiments, the double-stranded region of the RNA molecule does not contain any mismatch or bulge, and the two strands can be perfectly complementary to each other

in the double-stranded region. In another embodiment, the double-stranded region of the RNA molecule contains a mismatch and/or bulge.

[0162] In certain embodiments, the aiRNA includes at least one double-stranded region, and two ends independently selected from the group consisting of a 5'-overhang, a 3'-overhang, and a blunt end. In certain embodiments, the aiRNA includes at least one double-stranded region, a 5'-overhang, and a blunt end at the 3'-terminus (3'-blunt end). In certain embodiments, the aiRNA includes at least one double-stranded region, a 3'-overhang, and a blunt end at the 5'-terminus (5'-blunt end). In certain embodiments, the aiRNA includes at least one double-stranded region, a 3'-overhang, and a 5'-overhang.

[0163] In certain embodiments, the terminal overhang (including the 3'-overhang or 5'-overhang) includes 1-15 nucleotides. In certain embodiments, the terminal overhang includes 1-9 nucleotides. In certain embodiments, the terminal overhang includes 1-8 nucleotides. In certain embodiments, the terminal overhang includes 1-7 nucleotides. In certain embodiments, the terminal overhang includes 1-6 nucleotides. In certain embodiments, the terminal overhang includes 1-5 nucleotides. In certain embodiments, the terminal overhang includes 1, 2, 3, 4, 5, 6, or 7 nucleotides.

[0164] Thus, in certain embodiments, the 3'-overhang of the antisense strand of an RNA molecule of the present teachings includes 1-15 nucleotides. In certain embodiments, the 3'-overhang includes 1-9 nucleotides. In certain embodiments, the 3'-overhang includes 1-8 nucleotides. In certain embodiments, the 3'-overhang includes 1-7 nucleotides. In certain embodiments, the 3'-overhang includes 1-6 nucleotides. In certain embodiments, the 3'-overhang includes 1-5 nucleotides. In certain embodiments, the 3'-overhang includes 1 nucleotide. In certain embodiments, the 3'-overhang includes 2 nucleotides. In certain embodiments, the 3'-overhang includes 3 nucleotides. In certain embodiments, the 3'-overhang includes 4 nucleotides. In certain embodiments, the 3'-overhang includes 5 nucleotides.

[0165] In certain embodiments, the aiRNA includes a 5' overhang of 3 nucleotides and/or a 3' overhang of 3 nucleotides.

[0166] Thus, in certain embodiments, the 3'-terminus of the antisense strand of an RNA molecule of the present teachings includes 0-15 nucleotides. In certain embodiments, the 3'-terminus includes 0-9 nucleotides. In certain embodiments, the 3'-terminus includes 0-8

nucleotides. In certain embodiments, the 3'-terminus includes 0-7 nucleotides. In certain embodiments, the 3'-terminus includes 0-6 nucleotides. In certain embodiments, the 3'-terminus includes 0-5 nucleotides.

[0167] Thus, in certain embodiments, the 5'-overhang of the antisense strand of an RNA molecule of the present teachings includes 1-15 nucleotides. In certain embodiments, the 5'-overhang includes 1-9 nucleotides. In certain embodiments, the 5'-overhang includes 1-8 nucleotides. In certain embodiments, the 5'-overhang includes 1-7 nucleotides. In certain embodiments, the 5'-overhang includes 1-6 nucleotides. In certain embodiments, the 5'-overhang includes 1-5 nucleotides. In certain embodiments, the 5'-overhang includes 1 nucleotide. In certain embodiments, the 5'-overhang includes 2 nucleotides. In certain embodiments, the 5'-overhang includes 3 nucleotides. In certain embodiments, the 5'-overhang includes 4 nucleotides. In certain embodiments, the 5'-overhang includes 5 nucleotides.

[0168] Thus, in certain embodiments, the 5'-terminus of the antisense strand of an RNA molecule of the present teachings includes 0-15 nucleotides. In certain embodiments, the 5'-terminus includes 0-9 nucleotides. In certain embodiments, the 5'-terminus includes 0-8 nucleotides. In certain embodiments, the 5'-terminus includes 0-7 nucleotides. In certain embodiments, the 5'-terminus includes 0-6 nucleotides. In certain embodiments, the 5'-terminus includes 0-5 nucleotides.

[0169] Any region of the RNA molecule of the present teachings, including any terminal overhangs and gaps in between two double-stranded regions, can be stabilized against degradation, either through chemical modification or secondary structure. The RNA strands can have unmatched or imperfectly matched nucleotides. Each strand may have one or more nicks (a cut in the nucleic acid backbone), gaps (a fragmented strand with one or more missing nucleotides), and modified nucleotides or nucleotide analogues. Not only can any or all of the nucleotides in the RNA molecule be chemically modified, each strand may be conjugated with one or more moieties to enhance its functionality, for example, with moieties such as one or more peptides, antibodies, antibody fragments, aptamers, polymers and so on.

[0170] In certain embodiments, the first strand of an aiRNA of the present teachings can include ribonucleotides (or a RNA nucleotide). Each of the ribonucleotide in the certain embodiments can be an unmodified nucleotide or a modified nucleotide. In certain embodiments,

the 3'-overhang, the double-stranded region, or the 5'-overhang can include ribonucleotides. In certain embodiments, the 3'-overhang or the 3'-blunt end of the antisense strand can include ribonucleotides. In certain embodiments, the 3'-overhang of the antisense strand can include essentially ribonucleotides. For example, the 3'-overhang of the antisense strand can include ribonucleotides only.

[0171] In certain embodiments, the first strand of an aiRNA of the present teachings can include deoxyribonucleotides (or a DNA nucleotide). In certain embodiments, the 3'-overhang, the double-stranded region, or the 5'-overhang can include deoxyribonucleotides.

[0172] In certain embodiments, the second strand of an aiRNA of the present teachings can include ribonucleotides (or a DNA nucleotide). In certain embodiments, the second strand of an aiRNA of the present teachings can include deoxyribonucleotides (or a DNA nucleotide).

[0173] An aiRNA of the present teachings can include one or more modified or unmodified nucleotides. Accordingly, in certain embodiments, the aiRNA can include one or more unmodified nucleotides. In certain embodiments, the first strand of an aiRNA of the present teachings can include one or more unmodified nucleotides. In certain embodiments, the second strand of an aiRNA of the present teachings can include one or more unmodified nucleotides.

[0174] In certain embodiments, the first strand of an aiRNA of the present teachings includes one or more modified nucleotides. In certain embodiments, the first strand can include 1-21 modified nucleotides. In certain embodiments, the first strand can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 modified nucleotides. In certain embodiments, the first strand can include 1, 2, 3, 4, 5, 6, 7, 8, or 10 modified nucleotides. In certain embodiments, the first strand can include 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 modified nucleotides.

[0175] In many instances, any nucleotide in the first strand of an aiRNA of the present teachings can be a modified nucleotide. In certain embodiments, the first nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the second nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the third nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the fourth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the fifth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the sixth

nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the seventh nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the eighth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the ninth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the tenth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the eleventh nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the twelfth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the thirteenth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the fourteenth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the fifteenth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the sixteenth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the seventeenth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the eighteenth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the nineteenth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the twentieth nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the twenty-first nucleotide from the 5'-terminal nucleotide of the first strand is a modified nucleotide.

[0176] In certain embodiments, the first nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the second nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the third nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the fourth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the fifth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the sixth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the seventh nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the eighth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In

certain embodiments, the ninth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the tenth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the eleventh nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the twelfth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the thirteenth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the fourteenth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the fifteenth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the sixteenth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the seventeenth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the eighteenth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the nineteenth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the twentieth nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide. In certain embodiments, the twenty-first nucleotide from the 3'-terminal nucleotide of the first strand is a modified nucleotide.

[0177] In certain embodiments, the second strand of an aiRNA of the present teachings can include one or more modified nucleotides. In certain embodiments, the second strand can include 1-15 modified nucleotides. In certain embodiments, the second strand can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 modified nucleotides. In certain embodiments, the first strand can include 1, 2, 3, 4, 5, 6, 7, 8, or 10 modified nucleotides. In certain embodiments, the first strand can include 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 modified nucleotides.

[0178] In certain instances, any nucleotide in the second strand of an aiRNA of the present teachings can be a modified nucleotide. In certain embodiments, the first nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the second nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the third nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the fourth nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the fifth nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments,

the sixth nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the seventh nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the eighth nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the ninth nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the tenth nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the eleventh nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the twelfth nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the thirteenth nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the fourteenth nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the fifteenth nucleotide from the 5'-terminal nucleotide of the second strand is a modified nucleotide.

[0179] In certain embodiments, the first nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the second nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the third nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the fourth nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the fifth nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the sixth nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the seventh nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the eighth nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the ninth nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the tenth nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the eleventh nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the twelfth nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the thirteenth nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide. In certain embodiments, the fourteenth nucleotide from the 3'-terminal nucleotide of the second strand is a

modified nucleotide. In certain embodiments, the fifteenth nucleotide from the 3'-terminal nucleotide of the second strand is a modified nucleotide.

[0180] In certain embodiments, any one of the modified nucleotides in the first strand and the second strand of an aiRNA of the present teachings is independently modified at the nucleobase, the sugar, the internucleoside linkage, or a combination thereof. In certain embodiments, the modified nucleotide is modified at the nucleobase. In certain embodiments, the modified nucleotide is modified at the sugar. In certain embodiments, the modified nucleotide can include 2'-methoxy-sugar (i.e., where the sugar is methylated at its 2'-hydroxy, and the modified nucleotide in these certain embodiments sometimes being referred to as 2'-OMe modified nucleotides). In certain embodiments, the modified nucleotide is modified at the internucleoside linkage. Without being limited to any particular theory, aiRNAs including modified nucleotides (including 2'-OMe modified nucleotides) can have improved stability and activity in comparison to those with unmodified or less modified nucleotides.

[0181] In certain embodiments, the first strand or the antisense strand of an aiRNA of the present teachings is complementary to a target RNA. In certain embodiments, the 3'-overhang of the antisense strand is complementary to a target RNA. In certain embodiments, the double-stranded region of the antisense strand is complementary to a target RNA. In certain embodiments, the 5'-overhang of the antisense strand is complementary to a target RNA. In certain embodiments, the 3'-overhang and the double-stranded region of the antisense strand is complementary to a target RNA. In certain embodiments, the double-stranded region and the 5'-overhang is complementary to a target RNA. In certain embodiments, the complementarity between the antisense strand, or the 5'-overhang, the double-stranded region, or the 3'-overhang thereof, and the target RNA is partial complementarity, substantial complementarity, or perfect complementarity. In certain embodiments, the 3'-overhang of the antisense strand is partially complementary, substantially complementary, or perfectly complementary to the target RNA. In certain embodiments, the double-stranded region of the antisense strand is partially complementary, substantially complementary, or perfectly complementary to the target RNA. In certain embodiments, the 5'-overhang of the antisense strand is partially complementary, substantially complementary, or perfectly complementary to the target RNA.

[0182] In certain embodiments, one or more of the 3'-overhang, the double-stranded region, and the 5'-overhang of the antisense strand is independent from the target RNA. In certain embodiments, the 3'-overhang of the antisense strand is independent from the target RNA. In certain embodiments, the double-stranded region of the antisense strand is independent from the target RNA. In certain embodiments, the 3'-blunt end of the antisense strand is independent from the target RNA. In certain embodiments, the 5'-blunt end of the antisense strand is independent from the target RNA. In certain embodiments, the 5'-overhang of the antisense strand is independent from the target RNA.

[0183] In certain embodiments, the one or more of the 3'-overhang, the double-stranded region, and the 5'-overhang of the antisense strand that is independent from the target RNA can include A, U, or C. In certain embodiments, the one or more of the 3'-overhang, the double-stranded region, and the 5'-overhang of the antisense strand can include an "AA" motif, a "UU" motif, a "CC" motif, an "AU" motif, a "AC" motif, a "UA" motif, a "UC" motif, or a "CA" motif. In certain embodiments, the 5'-overhang or 5'-blunt end (or collectively the 5'-terminus) of the antisense strand can include an "AA" motif, a "UU" motif, a "CC" motif, a "AU" motif, a "AC" motif, a "UA" motif, a "UC" motif, or a "CA" motif. In certain embodiments, the 5'-overhang or 5'-blunt end of the antisense strand can include an "AA" motif. In certain embodiments, the 5'-overhang or 5'-blunt end of the antisense strand can include a "UU" motif. In certain embodiments, the 5'-overhang or 5'-blunt end of the antisense strand can include a "CC" motif. In certain embodiments, the 5'-overhang or 5'-blunt end of the antisense strand can include an "AU" motif. In certain embodiments, the 5'-overhang or 5'-blunt end of the antisense strand can include an "AC" motif. In certain embodiments, the 5'-overhang or 5'-blunt end of the antisense strand can include a "UA" motif. In certain embodiments, the 5'-overhang or 5'-blunt end of the antisense strand can include a "UC" motif. In certain embodiments, the 5'-overhang or 5'-blunt end of the antisense strand can include a "CA" motif.

[0184] In certain embodiments, the target RNA is an mRNA that encodes a ligand of PD-1. In certain embodiments, the target RNA is an mRNA that encodes PD-L1 or PD-L2. In certain embodiments, the target RNA is an mRNA that encodes PD-L1. In certain embodiments, the target nucleotide sequence is in an mRNA that encodes PD-L1.

[0185] In certain embodiments, an aiRNA of the present teachings can include a sense strand and an antisense strand, where the sense strand and the antisense strand form a double-stranded region, the sense strand is selected from SEQ ID NOs. 1-46, and the antisense strand is selected from SEQ ID NOs. 47-92, as follows:

SEQ ID NO.	Sense Strand Sequence*	SEQ ID NO.	Antisense Strand Sequence*
1	5'-CCUAUAUGUGGUAGA-3'	47	5'-AACUCUACCACAUAUAGGUCC-3'
2	5'-GUAGAGUAUGGUAGC-3'	48	5'-AAUGCUACCAUACUCUACCAC-3'
3	5'-GUUCAGCAUAGUAGC-3'	49	5'-AAAGCUACU AUGCUGAACCUU-3'
4	5'-UUGAAGGACCAGCUC-3'	50	5'-AAAGAGCUGGUCCUUAACAG-3'
5	5'-CACUUCAGAUACAG-3'	51	5'-AAUCUGUGAUCUGAAGUGCAG-3'
6	5'-CUUCAGAUACAGAU-3'	52	5'-AACAUUCUGUGAUCUGAAGUGC-3'
7	5'-CACAGAUGUGAAAUU-3'	53	5'-AACAAUUUCACAUCUGUGAUC-3'
8	5'-AUGUGAAAUUGCAGG-3'	54	5'-AAUCCUGCAAUUUCACAUCUG-3'
9	5'-CAGUCACCUCUGAAC-3'	55	5'-AAUGUUCAGAGGUGACUGGAU-3'
10	5'-CAUCUGGACAAGCAG-3'	56	5'-AAACUGCUUGUCCAGAUGACU-3'
11	5'-AUCUGGACAAGCAGU-3'	57	5'-AACACUGCUUGUCCAGAUGAC-3'
12	5'-AAGUCCUGAGUGGUA-3'	58	5'-AAUUACCACUCAGGACUUGAU-3'
13	5'-AGAGAGGAGAAGCUU-3'	59	5'-AAAAAGCUUCUCCUCUCUCUU-3'
14	5'-AGGAGAAGCUUUUCA-3'	60	5'-AAUUGAAAAGCUUCUCCUCUC-3'
15	5'-UGACCAGCACACUGA-3'	61	5'-AACUCAGUGUGCUGGUCACAU-3'
16	5'-ACUGAGAAUCAACAC-3'	62	5'-AAUGUGUUGAUUCUCAGUGUG-3'
17	5'-UUUCUACUGCACUUU-3'	63	5'-AAAAAAGUGCAGUAGAAAUC-3'
18	5'-GAUACAAACUCAAG-3'	64	5'-AAUCUUUGAGUUUGUAUCUUG-3'
19	5'-ACUCAAGAAGCAAA-3'	65	5'-AACUUUGCUUCUUUGAGUUUG-3'
20	5'-AAAGAAGCAAAGUGA-3'	66	5'-AAAUCACUUUGCUUCUUUGAG-3'
21	5'-GCAAAGUGAUACACA-3'	67	5'-AAAUGUGUAUCACUUUGCUUC-3'
22	5'-UGUGAAUGAUUUCUU-3'	68	5'-AAAAAGAAAUCAUUCACAACC-3'
23	5'-GUAGAUGUUAACAAU-3'	69	5'-AAAAAUUGUAACAUCUACUAC-3'
24	5'-CCUUUAUUUAACCCA-3'	70	5'-AAAUUGGGUUAUAUAAGGUGA-3'
25	5'-CUGAAGGUUCAGCAU-3'	71	5'-AAUAUGCUGAACCUUCAGGUC-3'
26	5'-GUUGUGGAUCCAGUC-3'	72	5'-AAUGACUGGAUCCACAACCAA-3'
27	5'-GAGAAGCUUUUCAU-3'	73	5'-ACAUUGAAAAGCUUCUCCUC-3'
28	5'-UAUUUAUGUAUGAGU-3'	74	5'-AAAACUCAUACAUAUAUACUG-3'
29	5'-CACAACAACUAAUGA-3'	75	5'-AACUCAUUAGUUGUUGUGUUG-3'
30	5'-UAAGAACAUUAUUCA-3'	76	5'-AAUUGAAUAAUGUUCUUAUCC-3'
31	5'-UUUUCUACUGCACUU-3'	77	5'-AAAAAGUGCAGUAGAAAUCU-3'
32	5'-UGUCAGGCUGAGGGC-3'	78	5'-AAAGCCCUCAGCCUGACAUGU-3'

SEQ ID NO.	Sense Strand Sequence*	SEQ ID NO.	Antisense Strand Sequence*
33	5'-UCUGGACAAGCAGUG-3'	79	5'-AAUCACUGCUUGUCCAGAUGA-3'
34	5'-AGAUGUGAAAUUGCA-3'	80	5'-AACUGCAAUUUCACAUCUGUG-3'
35	5'-CAGUGACCAUCAAGU-3'	81	5'-AAGACUUGAUGGUCACUGCUU-3'
36	5'-GAGAGAGGAGAAGCU-3'	82	5'-AAAAGCUUCUCCUCUCUCUUG-3'
37	5'-AGAGAGAGGAGAAGC-3'	83	5'-AAAGCUUCUCCUCUCUCUUGG-3'
38	5'-AGGAAGACCUGAAGG-3'	84	5'-AAACCUUCAGGUCUCCUCUC-3'
39	5'-CCACCACCAAUUGCA-3'	85	5'-AAUUGGAAUUGGUGGUGGUGG-3'
40	5'-GGAUCCAGUCACCUC-3'	86	5'-AAAGAGGUGACUGGAUCCACA-3'
41	5'-ACACAUUUGGAGGAG-3'	87	5'-AAUCUCCUCCAAAUGUGUAUC-3'
42	5'-AACUAAACUUGCUGC-3'	88	5'-AAAGCAGCAAGUUUAGUUUGG-3'
43	5'-CGGGACAGUAUUUAU-3'	89	5'-AACAUAAAUACUGUCCCGUUC-3'
44	5'-GUAUACAUUGGAAGC-3'	90	5'-AAUGCUUCCAAUGUAUACUUG-3'
45	5'-ACUAAACUUGCUGC-3'	91	5'-AAAAGCAGCAAGUUUAGUUUG-3'
46	5'-GUUGACCUAUCUUA-3'	92	5'-AAAUAAGAUUAGGUCAACCAG-3'

* G, C, U, and A stand for a ribonucleotide having guanine, cytosine, uracil, and adenine, respectively.

[0186] In certain embodiments, the aiRNA has a sense strand having a sequence of SEQ ID NO. 22 and an antisense strand having a sequence of SEQ ID NO. 68. This aiRNA in certain embodiments is referred to as aiRNA #22 or aiPD-L1 #22.

[0187] In certain embodiments, an aiRNA of the present teachings modulates the expression of one or more genes associated with a ligand of the programmed cell death-1 receptor (PD-1) on a lymphocyte. In certain embodiments, the lymphocyte is a T-cell, a T-helper cell, or a natural killer cell. In certain embodiments, the lymphocyte is a cytotoxic T-cell or CD8⁺ cell. In certain embodiments, the ligand of PD-1 is PD-L1 or PD-L2. In certain embodiments, the ligand is PD-L1. Accordingly, in certain embodiments, the aiRNA modulates the expression of PD-L1 or PD-L2. In certain embodiments, the aiRNA down-regulates, reduces, or inhibits the expression of PD-L1 or PD-L2. In certain embodiments, the aiRNA down-regulates, reduces, or inhibits the expression of PD-L1.

[0188] In certain embodiments, an aiRNA of the present teachings can therefore silence the gene associated with PD-L1 expression. Thus, in certain embodiments, the present teachings provide a PD-L1 inhibitor. As discussed herein in more details, in certain embodiments, the present

teachings provide a PD-L1-specific inhibitor. Solely for the convenience of discussion, aiRNAs of the present teachings sometimes can be referred to as aiPD-L1s (aiPD-L1 as the singular form).

[0189] In certain embodiments, an aiRNA of the present teachings down-regulates, reduces, or inhibits PD-L1 expression in cancer cells by mediating RNA interference or gene silencing in a sequence-specific manner. The specific gene silencing in many instances can improve the gene silencing and reduces undesired adverse events. The specific sequence in certain embodiments is referred to as a target site or target nucleotide sequence.

[0190] In certain embodiments, the target nucleotide sequence is a PD-L1 mRNA sequence or fragment thereof. In certain embodiments, a target nucleotide sequence of the present teachings can be chosen from the PD-L1 mRNA sequences of SEQ ID NO.: 93-138.

SEQ ID NO.	Target Nucleotide Sequence**
93	GGACCUAUAUGUGGUAGAGU ,
94	GUGGUAGAGUAUGGUAGCANA ,
95	AAGGUUCAGCAUAGUAGCU ,
96	CUGUUGAAGGACCAGCUCU ,
97	CUGCACUUCAGAUACAGAU ,
98	GCACUUCAGAUACAGAUUGU ,
99	GAUCACAGAUUGUGAAAUUGC ,
100	CAGAUUGUGAAAUUGCAGGAU ,
101	AUCCAGUCACCUCUGAACAU ,
102	AGUCAUCUGGACAAGCAGUG ,
103	GUCAUCUGGACAAGCAGUGA ,
104	AUCAAGUCCUGAGUGGUAA ,
105	AAGAGAGAGGAGAAGCUUUU ,
106	GAGAGGAGAAGCUUUUCAAU ,
107	AUGUGACCAGCACACUGAG ,
108	CACACUGAGAAUCAACACA ,
109	GAUUUUCUACUGCACUUUU ,
110	CAAGAUACAAACUCAAGA ,
111	CAAACUCAAGAAGCAAAGU ,
112	CUCAAAGAAGCAAAGUGAU ,
113	GAAGCAAAGUGAUACACAUUU ,
114	GGUUGUGAAUGAUUUCUUUU ,
115	GUAGUAGAUGUUACAAUUUU ,
116	UCACCUUUAUUUAACCCAUA ,

SEQ ID NO.	Target Nucleotide Sequence**
117	GACCUGAAGGUUCAGCAUANU ,
118	UUGGUUGUGGAUCCAGUCA ,
119	GAGGAGAAGCUUUUCA AUGU ,
120	CAGUAUUUAUGUAUGAGUUUU ,
121	CAACACAACAACUAAUGAGNU ,
122	GGAU AAGAACAUAUUAUCAAU ,
123	AGAUUUUCUACUGCACUUUU ,
124	ACAUGUCAGGCUGAGGGCU ,
125	UCAUCUGGACAAGCAGUGA ,
126	CACAGAUGUGAAAUUGCAG ,
127	AAGCAGUGACCAUCAAGUCNU ,
128	CAAGAGAGAGGAGAAGCUUUU ,
129	CCAAGAGAGAGGAGAAGCUUU ,
130	GAGAGGAAGACCUGAAGGUU ,
131	CCACCACCACCAAUCCAAU ,
132	UGUGGAUCCAGUCACCUCUNU ,
133	GAUACACAUUUGGAGGAGA ,
134	CCAAACUAAACUUGCUGCUU ,
135	GAACGGGACAGUAUUUAUGU ,
136	CAAGUAUACA UUGGAAGCAU ,
137	CAAACUAAACUUGCUGCUU , or
138	CUGGUUGACC UAAUCUUAUU .

** G, C, U, and A represent adenylate (adenosine 5'-monophosphate), guanylate (guanosine 5'-monophosphate), uridyate (uridine 5'-monophosphate) and cytidylate (cytidine 5'-monophosphate), respectively. N can be A, U, G or C

[0191] In certain embodiments, a target site for an aiRNA of the present teachings can be SEQ ID NO. 114.

[0192] In one aspect, the present teachings provide a composition including an RNA molecule of the present teachings. In certain embodiments, the composition can include a PD-L1 inhibitor. In certain embodiments, the composition can include an aiPD-L1. In certain embodiments, the composition can include an aiRNA having a sense strand and an antisense strand, where the sense strand and the antisense strand form a double-stranded region, the sense strand can be selected from SEQ ID NOs. 1-46, and the antisense strand can be the corresponding complementary nucleotide sequence chosen from SEQ ID NOs. 47-92. In certain embodiments, the composition

can include an aiRNA with a sense strand having a sequence of SEQ ID NO. 22 and an antisense strand having a sequence of SEQ ID NO. 68. In certain embodiments, this aiRNA is referred to as aiRNA #22 or aiPD-L1 #22.

[0193] In certain embodiments, the composition of the present teachings can include an excipient, carrier, or diluent. In certain embodiments, the composition of the present teachings can include a pharmaceutically acceptable excipient, carrier, or diluent.

[0194] In one aspect, the present teachings provide methods of modulating a gene expression in a cell. In certain embodiments, the cell can be a cancer cell. In certain embodiments, the gene encodes a ligand to the PD-1. In certain embodiments, the gene encodes PD-L1 or PD-L2. In certain embodiments, the gene encodes PD-L1. In certain embodiments, the method can include down-regulating, reducing, or inhibiting the expression of a ligand to the PD-1. In certain embodiments, the method can include down-regulating, reducing, or inhibiting the expression of PD-L1 or PD-L2. In certain embodiments, the method can include down-regulating, reducing, or inhibiting the expression of PD-L1.

[0195] In one aspect, the present teachings provide methods of modulating an interaction between a cell and a lymphocyte. In certain embodiments, the cell can be a cancer cell. In certain embodiments, the lymphocyte can be a T-cell, a T-helper cell, or a natural killer cell. In certain embodiments, the lymphocyte can be a cytotoxic T-cell or CD8⁺ cell. In certain embodiments, the interaction between a cell and a lymphocyte can be the binding of a ligand in the cell with a receptor in the lymphocyte. In certain embodiments, the interaction can be the binding of PD-L1 or PD-L2 with PD-1. In certain embodiments, the interaction can be the binding of PD-L1 with PD-1.

[0196] In certain embodiments, by silencing the gene that encodes PD-L1, the method reduces or inhibits the amount of PD-L1 mRNA and/or PD-L1 in the cell. In certain embodiments, as a result of the reduced amount of PD-L1 mRNA and/or PD-L1, the method reduces or inhibits the interaction between the cell and the lymphocyte. As a further result, in certain embodiments, the method activates or reactivates a lymphocyte (a T-cell, a cytotoxic T-cell, or a CD8⁺) so that the lymphocyte recognizes, attacks, and kills the cell. In certain embodiments, the method may also increase the infiltration of lymphocytes (T-cells, cytotoxic T-cells, or a CD8⁺s). In certain embodiments, the cell can be a cancer cell.

[0197] In another aspect, the present teachings provide an immunotherapy. In certain embodiments, the immunotherapy treats, prevents, or ameliorates a disorder in a subject. In certain embodiments, the immunotherapy can include providing a subject in need thereof with an aiRNA of the present teachings or a composition comprising an aiRNA of the present teachings. In certain embodiments, the immunotherapy can include providing a subject in need thereof with a therapeutically effective amount of an aiRNA of the present teachings or a therapeutically effective amount of a composition comprising an aiRNA of the present teachings.

[0198] In certain embodiments, the subject can be a mammal, including an animal or a human. In certain embodiments, the subject can be a human subject (or patient). In certain embodiments, the subject can be a mammalian cell. In certain embodiments, the subject can be a human cell. In certain embodiments, the subject can be a cancer cell. In certain embodiments, the subject can be diagnosed as positive for the expression of a ligand to PD-1. In certain embodiments, the subject can be PD-L1 positive. In certain embodiments, the subject can be PD-L2 positive. The diagnosis can be made according to methods generally known in the art. For example, it can be made based on the test of the relevant genetic material or an immuno-chemical method. An example of the diagnoses is discussed in Ohigashi et al., *Clin. Cancer Res.* 11(8):2947-2953 (2005) and the skilled artisan would understand that other methods can also be used to identify PD-L1 or PD-L2 positive subjects.

[0199] In certain embodiments, the disorder can be cancer. In certain embodiments, the cancer can be a hepatobiliary cancer (including liver cancer (including hepatocellular carcinoma or cholangiocarcinoma), gallbladder cancer, biliary cancer, or pancreatic cancer), gastrointestinal cancer, a urological cancer (including bladder cancer, prostate cancer, kidney cancer, testicular cancer, and the like), renal cell carcinoma, urothelial carcinoma, solid tumors, hepatocellular carcinoma, ovarian cancer, or cancers of the fallopian tubes. In certain embodiments, the cancer can be a hepatobiliary cancer (including liver cancer (including hepatocellular carcinoma or cholangiocarcinoma), gallbladder cancer, biliary cancer, or pancreatic cancer). In certain embodiments, the cancer can be gastrointestinal cancer. In certain embodiments, the cancer can be a urological cancer (including bladder cancer, prostate cancer, kidney cancer, testicular cancer, and the like), renal cell carcinoma, or urothelial carcinoma. In certain embodiments, the cancer can be a solid tumor. In certain embodiments, the solid tumor can be pancreatic cancer; bladder cancer; colorectal cancer; breast cancer; prostate cancer; renal cancer; hepatocellular cancer; lung

cancer; ovarian cancer; cervical cancer; gastric cancer; esophageal cancer; head and neck cancer; melanoma; neuroendocrine cancers; CNS cancers; brain tumors; bone cancer; or soft tissue sarcoma. In certain embodiments, the solid tumor can be lung cancer (non-small cell lung cancer, small-cell lung cancer), colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, or breast cancer.

[0200] In certain embodiments, the cancer can be acute myelogenous leukemia, astrocytoma, bladder cancer, bone cancer, brain cancer, breast cancer, chronic myelogenous leukemia, colon cancer, colorectal cancer, gastric cancer, gastrointestinal cancer, genitourinary cancer, glioblastoma, glioma, head and neck cancer, hepatoma, Hodgkin's lymphoma, Kaposi's sarcoma, lung cancer, melanoma, myeloproliferative disorders, non-Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, squamous cell carcinoma, or thyroid cancer.

[0201] Thus, in certain embodiments, the present teachings provide a method of treating a cancer in a subject where the method includes administering to a subject in need thereof a therapeutically effective amount of an aiRNA or a composition of the present teachings, where the cancer is hepatobiliary cancer (including liver cancer (including hepatocellular carcinoma or cholangiocarcinoma), gallbladder cancer, biliary cancer, or pancreatic cancer), gastrointestinal cancer, a urological cancer (including bladder cancer, prostate cancer, kidney cancer, testicular cancer, and the like), renal cell carcinoma, urothelial carcinoma, solid tumors, hepatocellular carcinoma, ovarian cancer, or cancers of the fallopian tubes. In some embodiments, the present teachings provide a method of treating a cancer in a subject where the method includes administering to a subject in need thereof a therapeutically effective amount of an aiRNA or a composition of the present teachings and the cancer is liver cancer, gallbladder cancer, biliary cancer, or pancreatic cancer. In certain embodiments, the present teachings provide a method of treating a cancer in a subject where the method includes administering to a subject in need thereof a therapeutically effective amount of an aiRNA or a composition of the present teachings and the cancer is gastrointestinal cancer, bladder cancer, prostate cancer, kidney cancer, testicular cancer, renal cell carcinoma, urothelial carcinoma, or hepatocellular carcinoma. In certain embodiments, the present teachings provide a method of treating a cancer in a subject where the method includes administering to a subject in need thereof a therapeutically effective amount of an aiRNA or a composition of the present teachings and the cancer is a solid tumor. In certain embodiments, the

present teachings provide a method of treating a cancer in a subject where the method includes administering to a subject in need thereof a therapeutically effective amount of an aiRNA or a composition of the present teachings and the cancer is pancreatic cancer; bladder cancer; colorectal cancer; breast cancer; prostate cancer; renal cancer; hepatocellular cancer; lung cancer; ovarian cancer; cervical cancer; gastric cancer; esophageal cancer; head and neck cancer; melanoma; neuroendocrine cancers; CNS cancers; brain tumors; bone cancer; or soft tissue sarcoma.

[0202] In certain embodiments, the present teachings provide a method of treating a cancer in a subject where the method includes administering to a cancer cell an aiRNA or a composition of the present teachings, where the cancer is hepatobiliary cancer (including liver cancer (including hepatocellular carcinoma or cholangiocarcinoma), gallbladder cancer, biliary cancer, or pancreatic cancer), gastrointestinal cancer, a urological cancer (including bladder cancer, prostate cancer, kidney cancer, testicular cancer, and the like), renal cell carcinoma, urothelial carcinoma, solid tumors, hepatocellular carcinoma, ovarian cancer, or cancers of the fallopian tubes. In certain embodiments, the present teachings provide a method of treating a cancer in a subject where the method includes administering to a cancer cell an aiRNA or a composition of the present teachings and the cancer is liver cancer, gallbladder cancer, biliary cancer, or pancreatic cancer. In certain embodiments, the present teachings provide a method of treating a cancer in a subject where the method includes administering to a cancer cell an aiRNA or a composition of the present teachings and the cancer is gastrointestinal cancer, bladder cancer, prostate cancer, kidney cancer, testicular cancer, renal cell carcinoma, urothelial carcinoma, or hepatocellular carcinoma. In certain embodiments, the present teachings provide a method of treating a cancer in a subject where the method includes administering to a cancer cell an aiRNA or a composition of the present teachings and the cancer is a solid tumor. In certain embodiments, the present teachings provide a method of treating a cancer in a subject where the method includes administering to a cancer cell an aiRNA or a composition of the present teachings and the cancer is pancreatic cancer; bladder cancer; colorectal cancer; breast cancer; prostate cancer; renal cancer; hepatocellular cancer; lung cancer; ovarian cancer; cervical cancer; gastric cancer; esophageal cancer; head and neck cancer; melanoma; neuroendocrine cancers; CNS cancers; brain tumors; bone cancer; or soft tissue sarcoma. The administering can be *in vitro* or *in vivo*. A person of ordinary skill in the art would understand that the method can be achieved by administering to the subject an aiRNA of the present teachings, a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable

solvate thereof, a prodrug thereof, or the like. In the case of prodrugs, the compound can be administered to a cancer cell when the prodrug is converted to the compound *in vivo*.

[0203] In certain embodiments, each of the cancers discussed herein can be advanced, recurrent, refractory to a standard cancer treatment, and/or metastatic. The standard cancer treatment can include surgery, chemotherapy, and/or radiation therapy.

EXAMPLES

[0204] The aiRNAs of the present teachings can be prepared using the methods generally known to the skilled artisans or those described in PCT Patent Application Publication No. WO2009029688, the entirety of which is incorporated herein by reference.

Example 1: Expansion of CMV-specific CTLs

[0205] HLA-typed PBMCs were purchase from Cellular Technology, Ltd. PBMCs were diluted at 5×10^6 cells/mL in culture medium (RPMI-1640 supplemented with 10% inactivated human serum, 50 μ M 2-mercaptoethanol), and seeded into 24-well plate (5×10^6 cells/mL/well). HLA-A*02:01 CMV pp65 peptide (NLVPMVATV) (IBA-Lifesciences, USA) was added to a final concentration of 5 μ M on day 0 as well as 25 IU/mL IL-2 and 5 ng/mL IL-15. The cultures were supplemented with fresh medium containing 10 μ M HLA-A*02:01 CMV pp65 peptide, 50 IU/mL IL-2 (R&D systems, USA) and 10 ng/mL IL-15 (R&D systems, USA) every 3-4 days. CD8⁺ T cells were isolated from PBMCs using a CD8⁺ T cell isolation kit (Miltenyi Biotec, Germany) according to the manufacturer's instructions.

Example 2: Transfection of PD-L1 and GFP aiRNA into luciferase-MDA-MB-231 cells

[0206] Luciferase expressing MDA-MB-231 cells (Luc-MDA-MB-231) were purchased from Cell Biolabs, Inc. and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% inactivated fetal calf serum (FCS). 24 h before transfection, the cells were seeded into 6-well plates (5×10^4 cells/2 mL/well). The cells were then transfected with PD-L1 aiRNA (aiPD-L1 #22) or GFP aiRNA using Lipofectamine® RNAiMAX (Thermo Fisher, USA) at 1 nM final concentration according to the manufacturer's instructions. The aiRNAs and RNAiMAX were incubated for 20 minutes in serum free OPTI-MEM (Thermo Fisher, USA), before being added to

the cells with culture medium. 48 hours after transfection, the cells were harvested using Accutase® cell detachment solution (Sigma Aldrich, USA). GFP aiRNA was used as a control. PD-L1 mRNA levels were measured by RT-PCR and PD-L1 protein expression was determined by flow cytometry.

[0207] As shown in FIGs. 2-4, 1 nM of aiPD-L1 #22 decreased PD-L1 mRNA in Luc-MDA-MB-231 cell line by 77.8% and aiPD-L1 #22 reduced the surface PD-L1 expression on Luc-MDA-MB-231 cell. FIG. 3 is a histogram and FIG. 4 shows the Mean fluorescent Intensity (C).

Example 3: Cytotoxic assay

[0208] aiPD-L1 #22 transfected Luc-MDA-MB-231 cells were incubated with or without HLA-A*02:01 CMV pp65 peptide (NLVPMVATV) for 2 hours in 37%, 5% CO₂ then washed with PBS twice. CMV peptide loaded or non-loaded Luc-MDA-MB-231 cells were plated into 96-well plates (2000 cells/well). CMV-specific CD8⁺ T cells were then added to the 96-well plates with an Effector: Target (E/T) ratio of 50:1 and further incubated for 24 hours. 10 µg/mL (final concentration) anti-Human PD-L1 Ab (Clone: MIH1, Functional grade purified) (Affymetrix eBioscience, USA) was used as a control. The intracellular luciferase activity of the Luc-MDA-MB-231 cells was then measured using a XenoLight D-Luciferin K⁺ salt (PerkinElmer, USA). The percent lysis was calculated as Luminescence of CMV peptide pulsed Luc-MDA-MB-231/ Luminescence of CMV peptide un-pulsed Luc-MDA-MB-231)*100.

[0209] As shown in FIG. 5, aiPD-L1 # 22 increased the antigen specific cytotoxicity of CD8⁺ T cells *in vitro* to a level equal to, if not greater than, the anti-human PDL1 antibody.

Example 4: Serum stability of 2'-OMe modified aiPD-L1

[0210] An unmodified aiPD-L1 or a 2'-OMe modified aiPD-L1 (with 10 modifications at both 3'- and 5'-termini in the first strand and 5 modifications at both 3'- and 5'-termini in the second strand) was incubated in 100% human serum for 3 hours. aiRNA degradation in each sample was then assessed by running a 15% PAGE gel. As shown in FIG. 6, the 2'-OMe modified aiPD-L1 was significantly more stable than the unmodified aiPD-L1.

Example 5: Potency of 2'-OMe modified aiPD-L1

[0211] The 2'-OMe modified aiPD-L1 of Example 4 was transfected into RKO cells at a concentration of 50 pM, 250 pM, and 500 pM. Western blot analysis of PD-L1 expression was conducted two days post transfection. Immunoblot analysis of total cell lysates was then performed using anti-PD-L1 and anti- β -actin antibodies. As shown in FIG. 7, the 2'-OMe modified aiPD-L1 was effective at inhibiting PD-L1 protein expression even at pM concentrations.

Example 6: Cytotoxicity of 2'-OMe modified aiPD-L1

[0212] To determine the cytotoxicity of the modified aiPD-L1, 1 nM of the 2'-OMe modified aiPD-L1 of Example 4 or a GFP control aiRNA was transfected into luciferase expressing MDA-MB-231 cells. CD8⁺ T cells expanded in a mixed lymphocyte-peptide cultures (MIPC) were isolated by MACS separation. Peptide pulsed/un-pulsed MDA-MB-231 and CD8⁺ T cells were then co-cultured overnight, and the number of viable cells was measured by luminescence.

[0213] As shown in FIG. 8, the 2'-OMe modified aiPD-L1 increased antigen-specific cytotoxicity of CD8⁺ T cells *in vitro*.

Example 7: Co-culture with aiPD-L1/TCR activator transfected breast cancer cells increases IL-2 expression in PD-1⁺ Jurkat cells treated with α CD28 antibody

[0214] AiScramble or aiPD-L1 RNA was transfected into MDA-MB-231 cells (human breast adenocarcinoma cell line; ATCC® HTB-26™) together with a TCR-Activator plasmid (BPS Bioscience). Jurkat T cells stably expressing PD-1 were co-cultured with the aiRNA transfected MDA-MB-231 cells and stimulated with α CD28 antibody for 24 hours. IL-2 expression was measured by ELISA.

[0215] FIG. 9 shows that co-cultured aiPD-L1 transfected MDA-MB-231 cells, but not aiScramble transfected MDA-MB-231 cells, restored α CD28 antibody induced IL-2 expression in PD-1⁺ Jurkat.

[0216] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the present teachings described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. An RNA molecule comprising
 - an antisense strand comprising 5'-terminal and 3'-terminal nucleotides that are 17, 18, 19, 20, or 21 nucleotides apart, and
 - a sense strand comprising a 5'-terminal nucleotide that is complementary to a nucleotide of the antisense strand other than its 3'-terminal nucleotide and a 3'-terminal nucleotide that is complementary to a nucleotide of the antisense strand,
 - wherein at least 14 nucleotides of the antisense strand are complementary with the sense strand, and
 - wherein at least 14 nucleotides of the antisense strand are colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.
2. The RNA molecule of claim 1, wherein at least 14 nucleotides of the antisense strand are colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163 or 164.
3. The RNA molecule of claim 1, wherein the antisense strand comprises the nucleotide sequence of SEQ ID NO. 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91 or 92.
4. The RNA molecule of claim 1, wherein the antisense strand comprises at least 7 nucleotides that are colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137 or 138.

5. The RNA molecule of claim 1, wherein at least 7 of the at least 14 nucleotides of the antisense strand are contiguous and colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.
6. The RNA molecule of claim 1, wherein at least 14 nucleotides of the sense strand are colinear with a target nucleotide sequence in SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.
7. The RNA molecule of claim 1, wherein at least 14 nucleotides of the sense strand are colinear with a target nucleotide sequence in SEQ ID NO. 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163 or 164.
8. The RNA molecule of claim 1, wherein the sense strand comprises a nucleotide sequence chosen from SEQ ID NO. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46.
9. The RNA molecule of claim 1, wherein 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 nucleotides of the antisense strand are not contiguous with the corresponding colinear complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.
10. The RNA molecule of claim 1, wherein 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 nucleotides of the antisense strand are not complementary to the corresponding colinear nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.
11. The RNA molecule of claim 1, wherein at least 14 nucleotides of the sense strand are contiguous and colinear with the corresponding complementary nucleotides of the antisense strand.

12. The RNA molecule of claim 1, wherein the 5'-terminal nucleotide of the sense strand is complementary to the first, second or third nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand.
13. The RNA molecule of claim 11, wherein the 3'-terminal nucleotide of the sense strand is complementary to the first, second or third nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand.
14. The RNA molecule of claim 1, wherein the 5'-terminal and 3'-terminal nucleotides of the sense strand are 13 nucleotides apart.
15. The RNA molecule of claim 13, wherein 19, 20 or 21 nucleotides of the antisense strand are colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.
16. The RNA molecule of claim 1, wherein the nucleotide sequence of the antisense strand that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34% or about 35%.
17. The RNA molecule of claim 1, wherein the nucleotide sequence of the antisense strand that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43% or about 44%.
18. The RNA molecule of claim 1, wherein the nucleotide sequence of the antisense strand that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57% or about 58%.

19. The RNA molecule of claim 1, wherein the nucleotide sequence of the antisense strand that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 33%.
20. Anti-PD-L1 aiRNAs 1-46.
21. The RNA molecule of any one of claims 1-20, wherein either the sense or antisense strand comprises at least one modified nucleotide or its analogue.
22. The RNA molecule of claim 21, wherein a 2'-OH group of the at least one modified ribonucleotide or its analogue is replaced by H or a 2'-O-methyl group.
23. The RNA molecule of claim 22, wherein the at least one modified nucleotide or its analogue is a sugar-, backbone-, and/or base-modified ribonucleotide.
24. The RNA molecule of claim 23, wherein the backbone-modified ribonucleotide comprises a modification in a phosphodiester linkage with another ribonucleotide.
25. The RNA molecule of claim 24, wherein the phosphodiester linkage is modified to comprise a nitrogen or a sulfur heteroatom.
26. The RNA molecule of claim 24, wherein the at least one modified nucleotide or its analogue comprises a phosphothioate group.
27. The RNA molecule of claim 24, wherein the at least one modified nucleotide or its analogue comprises inosine or a tritylated base.
28. The RNA molecule of claim 24, wherein the at least one modified nucleotide or its analogue is a sugar-modified ribonucleotide, wherein a 2'-OH group is replaced by H, OR, R, halo, SH, SR, NH₂, NHR, NR₂, or CN, and wherein each R is independently C1-C6 alkyl, alkenyl or alkynyl, and halo is F, Cl, Br, or I.
29. The RNA molecule of anyone of the preceding claims, wherein the 5'-terminal nucleotide and the first nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand

comprise an “AA” motif, a “UU” motif, a “CC” motif, a “AU” motif, a “AC” motif, a “UA” motif, a “UC” motif, or a “CA” motif.

30. The RNA molecule of anyone of the preceding claims, wherein the RNA molecule comprises a deoxyribonucleotide.
31. The RNA molecule of anyone of the preceding claims, wherein the first nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand is not dT.
32. The RNA molecule of anyone of the preceding claims, wherein silencing of a PD-L1 expressed nucleotide sequence by the RNA molecule is therapeutically more effective than an anti-PD-L1 antibody at enhancing tumor-specific T cell cytotoxicity.
33. The RNA molecule of anyone of the preceding claims, wherein administration of the RNA molecule into a subject in need thereof does not induce an interferon response.
34. A composition comprising the RNA molecule of claim 1.
35. The composition of claim 34, wherein the composition comprises nanoparticles having one or more PD-L1 aiRNAs.
36. A kit comprising the RNA molecule of claim 1 or the composition of claim 34.
37. An expression vector comprising a nucleic acid sequence encoding the RNA molecule of claim 1.
38. The expression vector of claim 37, wherein the expression vector is a viral, a eukaryotic, or a bacterial expression vector.
39. An isolated cell comprising the expression vector of claim 37.
40. An isolated cell comprising the RNA molecule of claim 1.
41. The cell of claim 40, wherein the cell is a mammalian, avian, insect, yeast or bacterial cell.

42. A method for treating a disease or condition comprising administering a therapeutically effective amount of the RNA molecule of any one of claims 1-33 to a subject in need thereof.
43. A method for treating, preventing, or ameliorating cancer in a subject comprising administering a therapeutically effective amount of the RNA molecule of any one of claims 1-33 to a subject in need thereof, wherein the cancer is an AIDS-Related cancer, a breast cancer, a cancer of the digestive/gastrointestinal tract, an endocrine and neuroendocrine cancer, a cancer of the eye, a genitourinary cancer, a germ cell cancer, a gynecologic cancer, a head and neck cancer, a hematologic cancer, a musculoskeletal cancer, a neurologic cancer, a respiratory/thoracic cancer, a skin cancer, a childhood cancer or a cancer of unknown primary.
44. The method of claim 42, wherein the RNA molecule is administered systemically or locally.
45. The method of claim 44, wherein the cancer is metastatic, recurrent or resistant to chemotherapy and/or radiation.

EXEMPLARY NO. 11 413828

[illegible]

EXXAMPLARY PD-22 41FMA8

[illegible]

~~XXXXXXXXXX~~ 17-08 XXXX XX
~~XXXXXXXXXX~~ 8 1 2002 5

EXEMPLARY PD-L1 aINRA5

PD-L1 mRNA SEQUENCE (SEQ ID NO: 156)														
aINRA #	SEQ ID NOs	C	A	G	A	U	A	C	A	A	C	U	C	A
18	18	G	A	U	A	C	A	A	G	A	A	G	A	A
64	64	G	A	U	A	C	A	A	G	A	A	G	A	A
19	19	G	A	U	A	C	A	A	G	A	A	G	A	A
65	65	G	A	U	A	C	A	A	G	A	A	G	A	A
20	20	G	A	U	A	C	A	A	G	A	A	G	A	A
66	66	G	A	U	A	C	A	A	G	A	A	G	A	A
PD-L1 mRNA SEQUENCE (SEQ ID NO: 157)														
aINRA #	SEQ ID NOs	C	A	G	A	U	A	C	A	A	C	U	C	A
21	21	G	A	U	A	C	A	A	G	A	A	G	A	A
67	67	G	A	U	A	C	A	A	G	A	A	G	A	A
41	41	G	A	U	A	C	A	A	G	A	A	G	A	A
87	87	G	A	U	A	C	A	A	G	A	A	G	A	A
PD-L1 mRNA SEQUENCE (SEQ ID NO: 158)														
aINRA #	SEQ ID NOs	C	A	G	A	U	A	C	A	A	C	U	C	A
43	43	G	A	U	A	C	A	A	G	A	A	G	A	A
89	89	G	A	U	A	C	A	A	G	A	A	G	A	A
28	28	G	A	U	A	C	A	A	G	A	A	G	A	A
74	74	G	A	U	A	C	A	A	G	A	A	G	A	A

FIG. 1A (ctd.)

EXEMPLARY PD-L1 mRNAs		SEQUENCE 1 (SEQ ID NO: 159)		SEQUENCE 2 (SEQ ID NO: 160)		SEQUENCE 3 (SEQ ID NO: 161)		SEQUENCE 4 (SEQ ID NO: 162)	
PD-L1 mRNA SEQUENCE (SEQ ID NO: 159)	SEQUENCE 1 (SEQ ID NO: 159)	PD-L1 mRNA SEQUENCE (SEQ ID NO: 160)	SEQUENCE 2 (SEQ ID NO: 160)	PD-L1 mRNA SEQUENCE (SEQ ID NO: 161)	SEQUENCE 3 (SEQ ID NO: 161)	PD-L1 mRNA SEQUENCE (SEQ ID NO: 162)	SEQUENCE 4 (SEQ ID NO: 162)	PD-L1 mRNA SEQUENCE (SEQ ID NO: 163)	SEQUENCE 5 (SEQ ID NO: 163)
ALPHA #	SEQ ID NOS	ALPHA #	SEQ ID NOS	ALPHA #	SEQ ID NOS	ALPHA #	SEQ ID NOS	ALPHA #	SEQ ID NOS
33	22	33	23	42	42	44	44	44	44
	68		69		88		90		90
G A G U G U G U G U G U G A A U G A U U U C U U U G A G A U A		U A U A U U G U A G U A G U A U G U A C A A U U U G U C C C A		U U G U C G C C A A A C U A A A C U U G C U G C U A A U G A U		A A C U A C A A G U A U A C A U U G G A A G C A U A A G A U C A		G U A U A C A U U G G A A G C	
15		15		15		15		15	
21		21		21		21		21	
30.0		30.0		30.0		30.0		30.0	
3		3		3		3		3	
3		3		3		3		3	

FIG. 1B

% G+C	Exemplary PD-L1 aiRNA #
23%-35%	17, 18, 19, 21, 22, 23, 24, 28, 29, 30, 31, 35, 44, 46
36%-44%	3, 7, 8, 12, 13, 14, 16, 20, 27, 34, 36, 41, 42, 43, 45
45%-57%	1, 2, 4, 5, 6, 9, 10, 11, 15, 25, 26, 38, 40, 32, 33, 37, 39

FIG. 1C

PD-L1 mRNA SEQUENCE (Transcript Variant 1: SEQ ID NO.: 166) (ACCESSION No.: NM 014143) PD-L1 CDS: 109-981		
(SEQ ID NO.: 139)		
1	<u>GGCGCAACGCUGAGCAGCUGGCGCGUCCCGCGCGGCCCCAGUUCUGCGCA</u>	50
51	GCUCCCCGAGGCUCGCGACCAGCGCGCUUCUGUCCGCCUGCAGGGCAUU	100
101	CCAGAAAG <u>AUG</u> AGGAUAUUUGCUGUCUUUAUAUUGCAUGACCUACUGGCAU	150
(SEQ ID NO.: 140)		
151	UUGCUGAACGCAUUUACUGUCACGGUCCCCA <u>AAGGACCUAUAUGUGGUAGA</u>	200
201	<u>GUAUGGUAGCAAUA</u> UGACAAUUGAAUGCAAUUCACAGUAGAAAAACAAU	250
(SEQ ID NO.: 141)		
251	UAGACCUGGCUGCACUAAUUGUCUAUUGGGAA <u>AAUGGAGGAUAAGAACA</u> UU	300
301	<u>AUUCAAUUUGUGCA</u> UGGAGAGGAAGACCUGAAGGUUCAGCAUAGUAGCUA	350
(SEQ ID NO.: 144)		
351	CAGACAGAGGGCCCCGGCUGUUGAAGGACCAGCUCUCCUGGGAAUUGCUG	400
401	CACUUCAGAUACAGAUUGUGAAAUUGCAGGAUCCAGGGGUGUACCGCUGC	450
451	AUGAUCAGCUAUGGUGGUGCCGACUACAAGCGAAUUACUGUGAAGUCAAA	500
(SEQ ID NO.: 147)		
501	UGCCCCAUACAACAAAAUCAACCAAGAAU <u>UUUGGUUGUGGAUCCAGUCA</u>	550
551	<u>CCUCUGAACAUGA</u> ACUGACAUGUCAGGCUGAGGGGUACCCCAAGGCGGAA	600
(SEQ ID NO.: 150)		
601	GUCAUCUGGACAAGCAG <u>UGACCAUCAAGUCCUGAGUGGUAAGACCACCA</u> C	650
651	CACCAAUUCCAAGAGAGAGGAGAAGCUUUUCAUUGUGACCAGCACACUGA	700
(SEQ ID NO.: 155)		
701	GAAUCAACACAACAAC <u>CUAUAGAGAUUUUCUACUGGCACUUUUAGGAGAU</u> UA	750
751	GAUCCUGAGGAAAACCAUACAGCUGAAUUGGUCAUCCCAAGAACUACCUCU	800
801	GGCACAUCCUCCAAAUUGAAAGGACUCACUUGGUAUUUCUGGGAGCCAUCU	850

FIG. 1C (ctd.)

FD-L1 mRNA SEQUENCE (Transcript Variant 1: SEQ ID NO.: 166) (ACCESSION No.: NM 014143) FD-L1 CDS: 109-981		
851	UAUUAUGCCUUGGUGUAGCACUGACAUUCAUCUUCGCUUAAGAAAAGGG	900
(SEQ ID NO.: 156)		
901	AGAAUGAUGGAUGUGAAAAAUGUGGCAUCC <u>CAAGAUACAAACUCAAGAA</u>	950
951	<u>GCAAAGUGAUACA</u> CAUUUGGAGGAGACGUAAUCCAGCAUUGGAACUUCUG	1000
[STOP CODON]		
1001	AUCUCCAAGCAGGGAUUCUCAACCUGUGGUUAGGGGUUCAUCGGGGCUG	1050
1051	AGCGUGACAAGAGGAAGGAAUGGGCCCCGUGGGAUCCAGGCAAUGUGGGAC	1100
1101	UUAAAAGGCCCAAGCACUGAAAAUGGAACCUGGCGAAAGCAGAGGAGGAG	1150
1151	AAUGAAGAAAGAUGGAGUCAAAACAGGGAGCCUGGAGGGAGACCUUGAUAC	1200
1201	UUUCAAAUGCCUGAGGGGCUCAUCCGACGCCUGUGACAGGGAGAAAGGAUA	1250
1251	CUUCUGAACAAGGAGCCUCCAAGCAAAUCAUCCAUUGCUCAUCCUAGGAA	1300
1301	GACGGGUUGAGAAUCCCUAAUUUGAGGGUCAGUUCUUGCAGAAGUGCCCU	1350
1351	UUGCCUCCACUCAUUGCCUCAUUUUGUUUUCUGCAUGACUGAGAGUCUCA	1400
(SEQ ID NO.: 158)		
1401	GUGUUG <u>GAAACGGGACAGUAUUUAUGUAUGAGUUUUCCU</u> AUUUAUUUUGA	1450
1451	GUCUGUGAGGUCUUCUUGUCAUGUGAGUGUGGUUGUGAAUGAUUUCUUUU	1500
(SEQ ID NO.: 161)		
1501	GAAGAUAAUUGUAGUAGAUGUUACAAUUUUGUCGCCAAACUAAACUUGC	1550
1551	<u>UGCUGAAUGAU</u> UUGUCACAUUCUAGUAAAACAUGGAGUAUUUGUAAGGUG	1600
1601	CUUGGUCUCCUCUAUAACUACAAGUAUACAUUGGAAGCAUAAAGAUCAAA	1650
(SEQ ID NO.: 164)		
1651	CCGUUGGUUGCAUAGGAUGUCACCUUUUAUUUAACCCAUUAAUACUCUGGU	1700

FIG. 1C (ctd.)

PD-L1 mRNA SEQUENCE (Transcript Variant 1: SEQ ID NO.: 166) (ACCESSION No.: NM 014143) PD-L1 CDS: 109-981		
1701	UGACC UAAUCUUAUUCUCAGACC UCAAGUGUCUGUGCAGUAUCUGU UCCA	1750
1751	UUUAAAUAUCAGCUUUACAABUAUGUGGUAGCCUACACACAUA AUCUCAU	1800
1801	UUCAUCGCUGUAACCA CCCGUGUUGUGAUA ACCACUAUUAUUUUAC CCCAUC	1850
1851	GUACAGCUGAGGAAGCAAACAGAUUAAGUAACUUGCCCAAACCAGU AAAU	1900
1901	AGCAGACCUCAGACUGCCACCCACUGUCCUUUUUAUAUA CAAUUUACAGC	1950
1951	UAUAUUUUACUUUAAGCAAUUCUUUUUAUUCAAAAACCAUUUAUUAAGUGC	2000
2001	CCUUGCAAUAUCAAUCCGUGUGCCAGGCAUUGAAUCUACAGAUGUGAGCA	2050
2051	AGACAAAGUACCUGUCCUCAAGGAGCUCAUAGUAUAUAUGAGGAGAUUAAC	2100
2101	AAGAAAUGUAUUUAUUA CAAUUUAGUCCAGUGUCAUAGCAUAAGGAUGAU	2150
2151	GGGAGGGGAAAACCCGAGCAGUGUUGCCAAGAGGAGGAAAUAGGCCAAUG	2200
2201	UGGUCUGGGACGGUUGGAUAUACUUA AAACAUCUUAAUAUAUCAGAGUAAUU	2250
2251	UUCAUUUACAAAGAGAGGUCGGUACUUA AAAAUAACCCUGAAAAUAACAC	2300
2301	UGGAUUUCCUUUUUCUAGCAUUAUAUUUAU UCCUGAUUUGCCUUUGCCAUU	2350
2351	UAAUCUAAUGCUUGUUUAUAUAGUGUCUGGUAUUGUUUAACAGUUCUGUC	2400
2401	UUUUCUAUUUAAAUGCCACUAAAUUUUAAAUUCAUACCUUUCCAUGAUUC	2450
2451	AAAAUUCAAAAGAUC CCAUGGGAGAU GGUUGGAAAUCUCCACUUCAUCC	2500
2501	UCCAAGCCAUUCAAGUUUCCUUUCCAGAAGCAACUGCUACUGCCUUUCAU	2550
2551	UCAUAUGUUCUUCUAAAGAUAGUCUACAUUUGGAAAUGUAUGUUA AAAGC	2600
2601	ACGUUUUUUAAAAUUUUUUUCCUAAAUAGUAACACAUGUAUGGUCUGCU	2650

FIG. 1C (ctd.)

PD-L1 mRNA SEQUENCE (Transcript Variant 1: SEQ ID NO.: 166) (ACCESSION No.: NM 014143) PD-L1 CDS: 109-981		
2651	GUGUACUUUGCUAUUUUUUAUUUUUUAGUGUUUCUUAUAUAGCAGAUGG	2700
2701	AAUGAAUUUGAAGUUCCTCAGGGCUGAGGAGCCAUGCCUUCUUUGUUUUCUA	2750
2751	AGUUUUCUUUUCCCAUAGCUUUUCCAUAUUCUUUCAUAUGAUCCAGUAUAUG	2800
2801	UUAAAUAUGUCCUACAUAUACAUAUUAGACAACCAUCCAUUUGUUAAGUAUU	2850
2851	UGCUCUAGGACAGAGUUUGGAUUUGUUUAUGUUUGCUCAAAAGGAGACCC	2900
2901	AUGGGCUCUCCAGGGUGCACUGAGUCAAUUCUAGUCCUAAAAAGCAAUCUU	2950
2951	AUUAAUUAACUCUGUAUGACAGAAUCAUSUCUGGAACUUUUGUUUUCUGCU	3000
3001	UUCUGUCAAGUAUAAACUUCACUUUGAUGCUGUACUUGCAAAAUACACAUU	3050
3051	UUCUUUCUGGAAAUUCCGGCAGUGUACCUUGACUGCUAGCUACCCUGUGC	3100
3101	CAGAAAAGCCUCAUUCGUUGUGCUUGAACCCUUGAAUGCCACCAGCUGUC	3150
3151	AUCACUACACAGCCCUCCUAAGAGGCUUCCUGGAGGUUUCGAGAUUCAGA	3200
3201	UGCCCUGGGAGAUCCAGAGUUUCCUUUCCCUUUGGCCAUAUUCUGGUG	3250
3251	UCAAUGACAAGGAGUACCUUGGCUUUGCCACAUGUCAAGGCUGAAGAAAC	3300
3301	AGUGUCUCCAACAGAGCUCCUUGUGUUUAUCUGUUUGUACAUGUGCAUUUG	3350
3351	UACAGUAAUUGGUGUGACAGUGUUCUUUGUGUGAAUUACAGGCAAGAAUU	3400
3401	GUGGCUGAGCAAGGCACAUAGUCUACUCAGUCUAUUCUUAAGUCCUAACU	3450
3451	CCUCCUUGUGGUGUUGGAUUUGUAAGGCACUUUAUCCCUUUUGUCUCAUG	3500
3501	UUUCAUCGUAAAUGGCAUAGGCAGAGAUGAUACCUAAAUUCUGCAUUUGAU	3550
3551	UGUCACUUUUUGUACCUGCAUUAUUUAAUAAAAUAUUCUUAUUUUUUUU	3600

FIG. 1C (ctd.)

PD-L1 mRNA SEQUENCE (Transcript Variant 1: SEQ ID NO.: 166) (ACCESSION No.: NM 014143) PD-L1 CDS: 109-981		
3601	GUUACUUGGUACACCAGCAUGUCCAUUUUCUUGUUUAUUUUUGUGUUUAAU	3650
SEQ ID NO.: 165		
3651	AAAAUGUUCAGUUUAACAUCCAGUGGAGAAAGUUAAAAA	3691

FIG. 1D (ctd.)

FD-L1 mRNA SEQUENCES (ACCESSION No.: NM 014143)	FD-L1 mRNA sequence	SEQ ID NO.	Exemplary FD-L1 airnas
451 AUGAUCAGCUAUGUGUGGUCGACUACACAGCAUUAUACUGGAAAGUCA 500			
501 UGCCCCAUACAACAAAUCAACCAAGCAAUUUUGUGUGGGAUCCAGUCA 550 (SEQ ID NO.: 147)			
551 CCUCUGAACAUGA 563			
(SEQ ID NO.: 147) 531 UUUGUGUGGGAUCCAGUCA 550			
551 CCUCUGAACAUGAUGUGACAUUGUCAGGCGGAGAGGCUACCCCAAGGCGAAA 600	531-649	170	9,10,11, 12,26,32, 33,35,40
601 GUCAUCUGGACAAAGCAGUGACCAUACGUCUGAGUGGUAGGCCACCA 649 (SEQ ID NO.: 150)			
(SEQ ID NO.: 150) 617 GUGACCAUACAGUCGUGAGUGGUAGGCCACCA 650			
651 CACCAAUUCCAGACAGACAGAGAGCUUUUCAAUGUGACCAAGCACACUGA 700			
701 GAUCACACACACACUAUGACAUUUUUUUAUUGGACUUUUUAGGAGAUUA 750			
751 GAUCCUGAGGAAACCAUACAGUUGAUAUGGUCUAUUCUCCAGACUACUCCU 800			
801 GGCACAGUCCUCCAAUUGAAAGGACUACAUGGUAUAUUCUGGGAGGCUAUCU 850			
851 UAUUAUGCCUUGGUGAGCACUGACAUUACUCCUCCUUUAAGAAAAAGGG 900			
901 AGAAUGAGUGGAGUGGAAAAAUAUGUGGCAUUCACAGUACAAACUCAAAGA 950 (SEQ ID NO.: 156)	617-963	171	12,39,13, 36,37,27, 14,15,16, 29,31,17, 18,19,20

FIG. 1D (ctd.)

PD-L1 mRNA SEQUENCES (ACCESSION No.: NM 014143)	PD-L1 mRNA sequence	SEQ ID NO.	Exemplary PD-L1 siRNAs
951 <u>GCAAAGUGAUACA</u> 963			
(SEQ ID NO.: 156) 931 <u>CARGAUNCARACUCARAGAA</u> 950			
951 <u>GCAAAGUGAUACA</u> CAUUUUGAGGAGACGUAAUCCAGCAUUGGAAACUUCUG 1000			
1001 AUCUUCAAGCAGGGAUUCUACACUUGGUUUUUGGGGUUCAUUGGGGUCUG 1050			
1051 AGCGUGACACAGAGGAAATGAAUUGGGCCCGUGGAUGCAGGCAUUGGGAC 1100			
1101 UUAAGAGGCCCCAGCACUAGAAAUAUUGAACUUGGGGAAAGCAGAGAGGAGAG 1150			
1151 AAUGAAGAAAGAUUGAGAUUAAACAGGAGAGCCUUGAGGAGAGACCUUGAUAC 1200			
1201 UUUCAAUUGCCUGAGGGGCUCAUCUAGACUCCUUGACAGGGCAGAAAGGAUA 1250			
1251 CUUCUGAACACAGGAGGCUUCCAAAGCAAAUACAUCUUAUUGGUCAUCCUAGGAA 1300			
1301 GACGGGUGAGAAUCCCUAAUUUGAGGGUACAGUUCUUGCAGAGAGUCCU 1350			
1351 UUGCCUCCACUACAUGGCCUCAAUUUUUUUUUUGCAUCACUGACAGUCUCA 1400			
(SEQ ID NO.: 158) 1401 <u>GAACGGGACAGUAUUUUAUGAGGAGUUUUUCCU</u> 1439			
(SEQ ID NO.: 158)			
1407 <u>GAACGGGACAGUAUUUUAUGAGGAGUUUUUCCU</u> AAUUUUUUUUGA 1450			
1451 GUCUGGAGGGUUCUUGUUGUAGUUGAGUUGGUGGUGGUGGAGUAUUAUUUUU 1500			
	1407-1439	173	43, 28, 22, 23, 42, 45, 44, 46

FIG. 1D (ctd.)

FD-L1 mRNA SEQUENCES (ACCESSION No.: NM 014143)		FD-L1 mRNA sequence	SEQ ID NO.	Exemplary FD-L1 airnas
1501	GAAGAAUUAUUGUAGUAGUUAACAUAUUUUGUGCCCAACUAAACUUGG	1550		
1551	UGCUUAAUGAUUUUGGUCACAUUCUAGUUAUAAACAUGGAGUAUUUGUAGGUG	1600		
1601	CUUGGUCUCCUUAUAACUACAAAGUAAGUAUACAUUGGAAAGCAUAAAGACAAA	1650		
1651	CCGUGGUGGCAUAGGAGUGUACCUUUUUUUUUAACCCAUUAUUAUACUCUGGU	1700		
1701	UGACCUAAUCUUAUUCUCAGAGACCUCAAGUUGUCUUGGACGUAUCUUGUCCA	1750		
1651	CCGUGGUGGCAUAGGAGUGUACCUUUUUUUUUAACCCAUUAUUAUACUUGGU	1700		
1701	UGACCUAAUCUUAUUCUCAGAGACCUCAAGUUGUCUUGGACGUAUCUUGUCCA	1750		
1701	UGACCUAAUCUUAUUCUCAGAGACCUCAAGUUGUCUUGGACGUAUCUUGUCCA	1750		
1751	UUUAAAUAACACGCUUUAACAAUUAUUGUGUGAGCCUACACACAAUAUCUCAU	1800		
1801	UUCAUCCUUGUAAACCAACCUUGUUGUGUAUUAUCCACUAUUAUUUUUACCCAU	1850		
1851	GUACAGCUCAGGAGACCAAGCAGAUUAUUAAGUUAACUUGCCCAACCCAGUAAAU	1900		
1901	AGCAGACCUAGACUGCCACCCACCCACUUGCCUUUUUAUAUAACAUUUUACAGC	1950		
1951	UAUAUUUUUACUUUAAGCAUUCUUUUUAUUAUUAUUAUUAUUAUUAUUAUUAU	2000		
2001	CCUUGCAAUUAUCAAUCCGUGUGCCAGGCAUUGUAUUAUUAUUAUUAUUAUUAU	2050		
2051	AGACAAAGUACCUUGUCCUACAGGAGCUCUUAUUAUUAUUAUUAUUAUUAUUAU	2100		
2101	AAGAAAUUGUAUUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAU	2150		
2151	GGAGGGGGAUAAACUUGAGGAGGUGUUGCCACAGGAGGAAAUUAUAGGCCAAUG	2200		

FIG. 1D (ctd.)

FD-L1 mRNA SEQUENCES (ACCESSION No.: NM 014143)		FD-L1 mRNA sequence	SEQ ID NO.	Exemplary FD-L1 siRNAs
2201	UGGUCUGGACCGGUUGGAUAUACUUAACACUUCUUAUAUACAGAGUAUU	2250		
2251	UUCAUUUACAAAAGAGAGGUGCGUAUUUAAAUAUACCCUGAATAAUAACAC	2300		
2301	UGGAUUUCCUUUUCUAGCAUUUAUUUAUUUCCUGAUUUGCCUUUGCCAUU	2350		
2351	UAAUCUAAUGCCUUGUUUAUUAUAGUGUCUGGUUAUUGUUUAACAGUUCUUC	2400		
2401	UUUUCUAUUUAAAGCCACUAAMUUUUUAAAUUUCAAUACCUUCCAUAGAUUC	2450		
2451	AAAUUUCAAAGAGUCCCAUGGAGAGAGUGGUUGGAAAAGUCCUCCACUUCAUCC	2500		
2501	UCCAGGCCAUUCAAAGUUUCCUUUCCAGAGAGCAUGCUACUGCCUUUUAU	2550		
2551	UCAUAUGUUUCCUUAAGAUAGUAGUCCACAUUUGGAAAAGUAUGUUAAAAGC	2600		
2601	ACGUUUUUUUAAAAGUUUUUUUCCUAAAUAUAGAAACACAUUGUUGUCUGU	2650		
2651	GUGUACUUUGCUUUUUUUUAUUUAUUUUAAGUUUUUUUUUAUAUAGCAGAGG	2700		
2701	AUUGAAUUUGAAGUUGCCAGGGGCUAGAGAUCCAUUCCUUCUUUGUUUUUA	2750		
2751	AGUUUUUUUUUCCCAUAGCUUUUUCAUUUUAUUUUUUUAUAGAUCCAGUAUAG	2800		
2801	UUAAAUUAUUUCCUUAUACUAUUUUAUAGACACACCAUUAUUUGUUUAAGUAUU	2850		
2851	UGCUUUAAGGACAGAGUUUGGAUUUGUUUUUAUUUUUUUUUUUUUUUUUUUU	2900		
2901	AUGGGCUUCCUCCAGGUGGACUGAGUCAAUUUAUAGUCCUAAAAGCAAUUUU	2950		
2951	AUUUUUAUUAUUAUAGACAGAAUUAUUGUUGGACUUUUUUUUUUUUUUUUUU	3000		

FIG. 1D (ctd.)

PD-L1 mRNA sequence	SEQ ID NO.	Exemplary PD-L1 siRNAs
PD-L1 mRNA SEQUENCES (ACCESSION No.: NM 014143) 3001 UUCGUCACGUAUAAACUUCACUUGGUAUGGUAUACUUGCAAAAUACAAU 3050 3051 UUCUUUUCUGGAAAUUUCGGGACAGUGGUAUACUUGGUAUACUUGGUC 3100 3101 CAGAAAAGCCUUAUUCGUGGUGGUAUACUUGGUAUACUUGGUC 3150 3151 AUCACUACACAGCCUUGUAACAGGCUUUCUUGGUAUACUUGGUC 3200 3201 UGCUUUGGUAUACUUGGUAUACUUGGUAUACUUGGUC 3250 3251 UCAUUGACAGGAGUACUUGGUAUACUUGGUAUACUUGGUC 3300 3301 AGUUGUCCACAGAGUACUUGGUAUACUUGGUAUACUUGGUC 3350 3351 UACAGUAUUGGUAUACUUGGUAUACUUGGUAUACUUGGUC 3400 3401 GUGGCUAGCAAGGUAUACUUGGUAUACUUGGUAUACUUGGUC 3450 3451 CUGGCUAGCAAGGUAUACUUGGUAUACUUGGUAUACUUGGUC 3500 3501 UUCUUGGUAUACUUGGUAUACUUGGUAUACUUGGUC 3550 3551 UGCUUUGGUAUACUUGGUAUACUUGGUAUACUUGGUC 3600 3601 GUAUUGGUAUACUUGGUAUACUUGGUAUACUUGGUC 3650 (SEQ ID NO.: 165) 3651 AAAAUUUCAGUUAUACUUGGUAUACUUGGUAUACUUGGUC 3689		

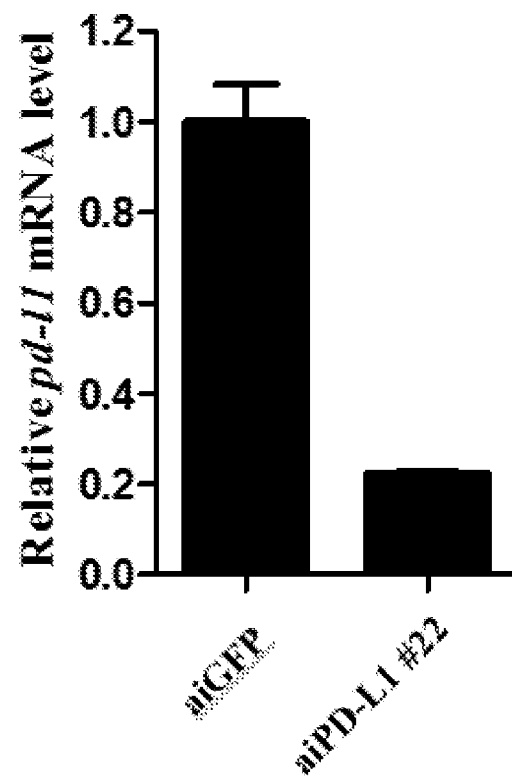


FIG. 2

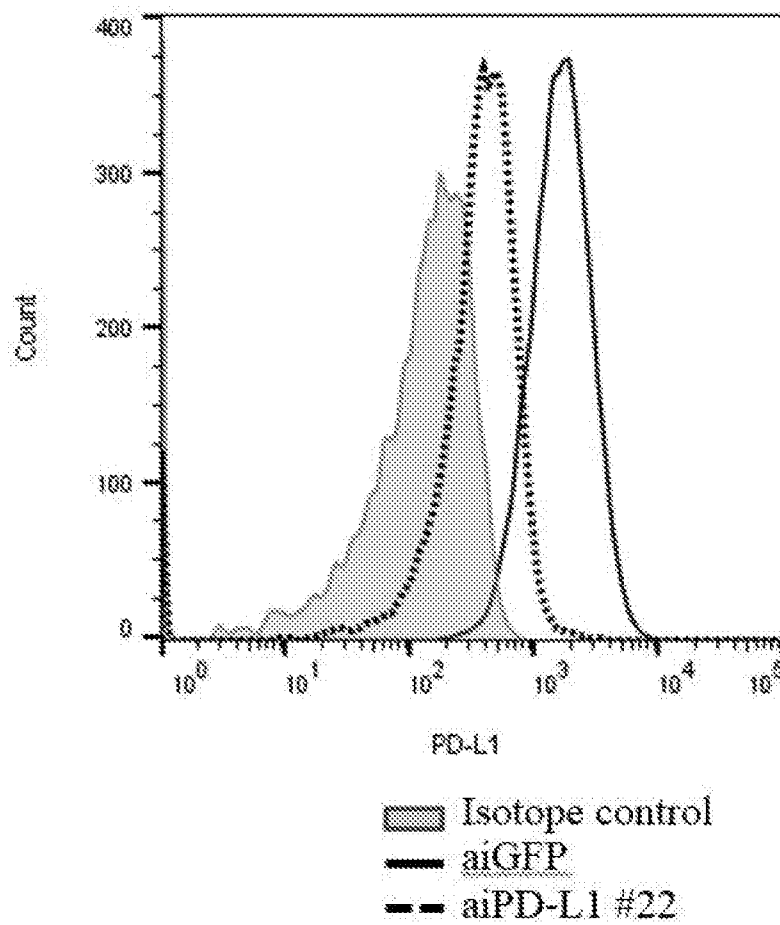


FIG. 3

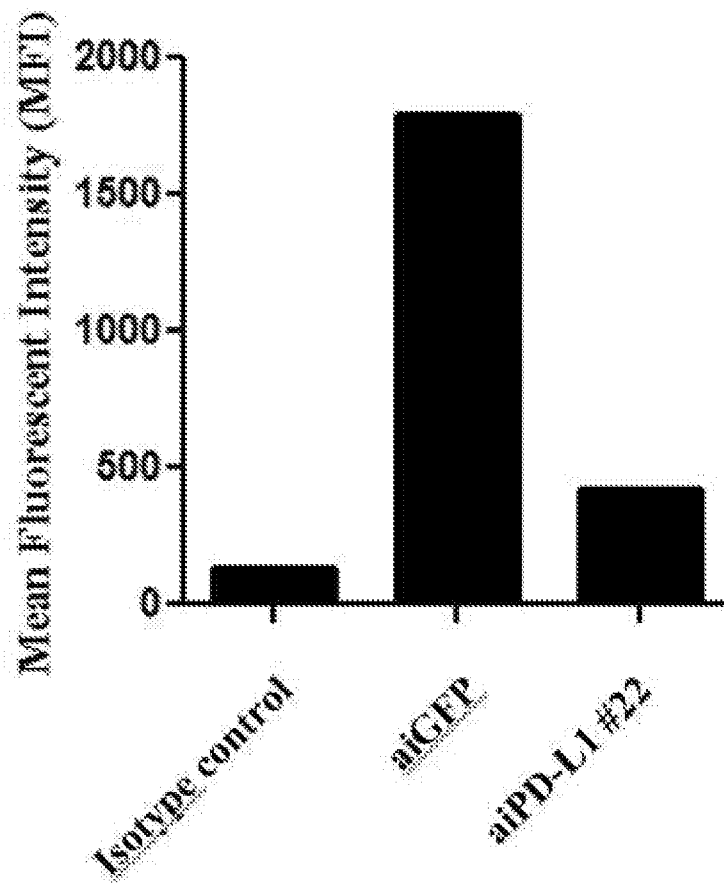


FIG. 4

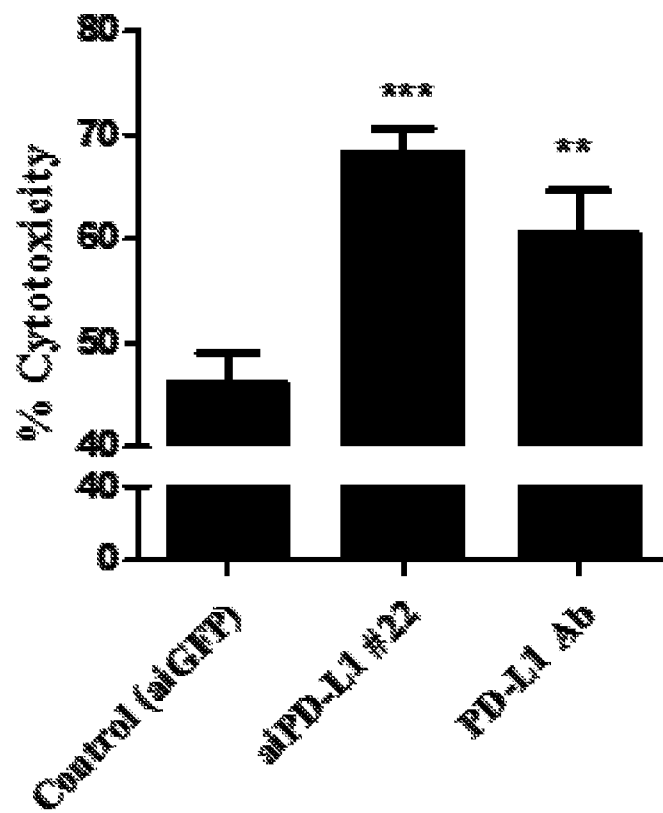


FIG. 5

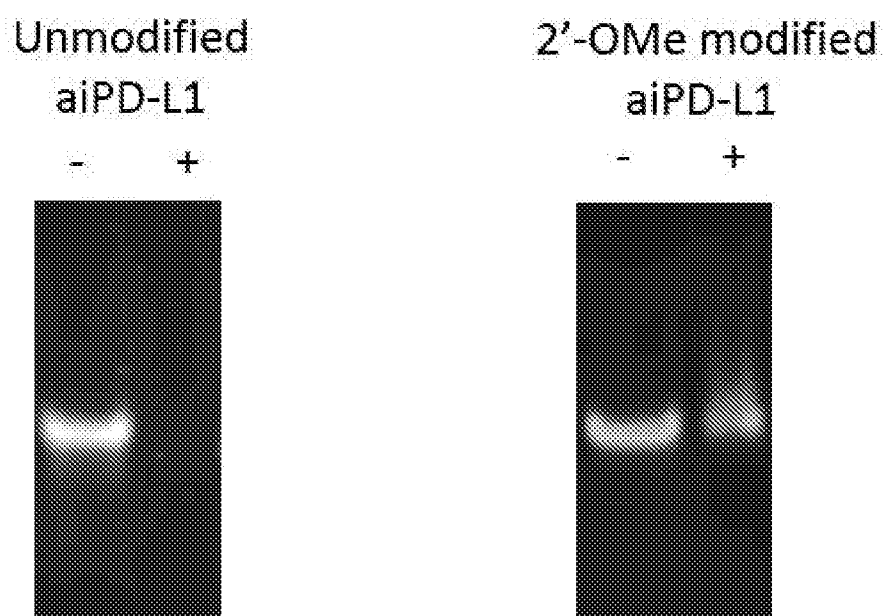


FIG. 6

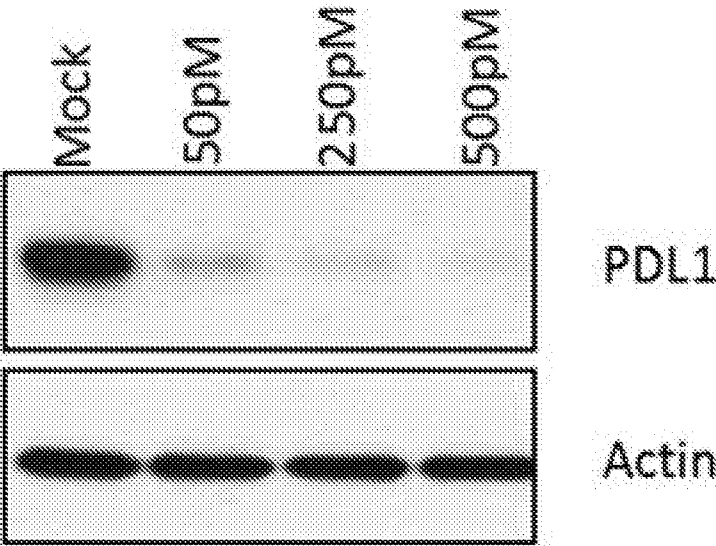


FIG. 7

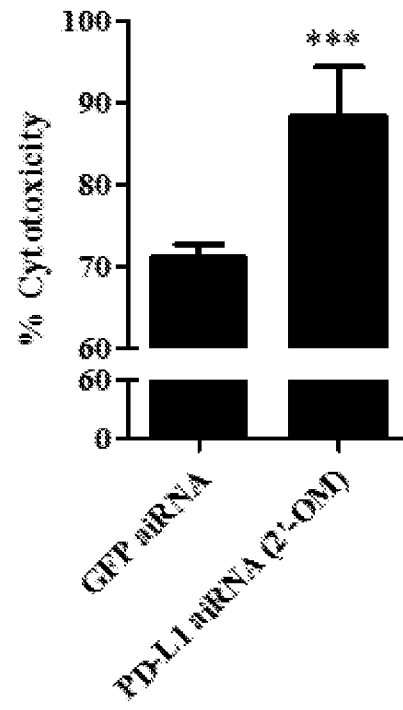


FIG. 8

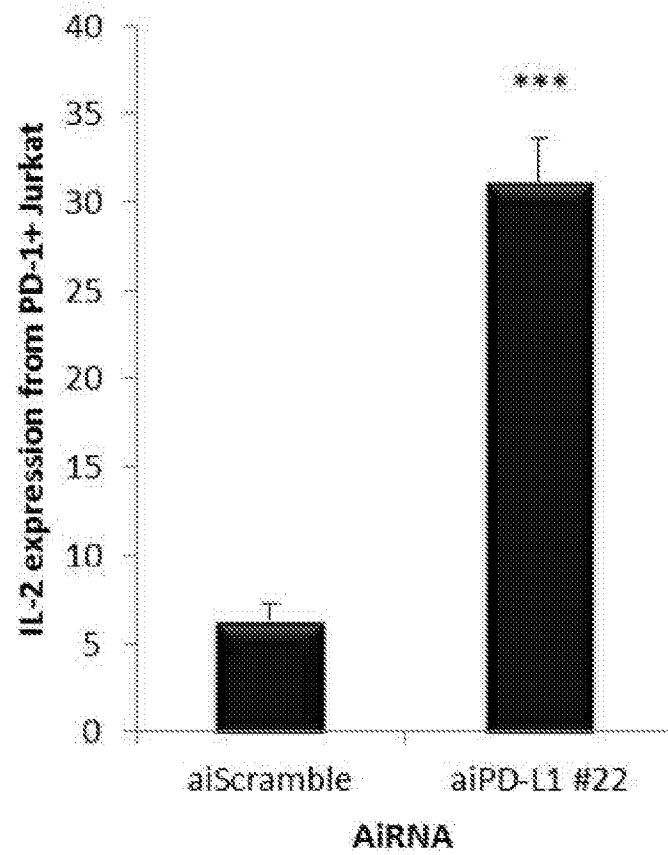


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/064951

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

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

B. FIELDS SEARCHED

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

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2005/007855 A2 (SIRNA THERAPEUTICS, INC. [US]; USMAN NASSIM [US]; MCSWIGGEN JAMES [US]) 27 January 2005 (2005-01-27) page 151; sequences 8, 94 page 152 - page 158 the whole document	1-45
Y	WO 2009/029688 A2 (BOSTON BIOMEDICAL, INC. [US]; LI CHIANG JIA; SUN XIANGAO; ROGOFF HARRY) 5 March 2009 (2009-03-05) examples ----- -/--	1-45



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

2 May 2017

Date of mailing of the international search report

11/05/2017

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040,
 Fax: (+31-70) 340-3016

Authorized officer

Macchia, Giovanni

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/064951

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TAKANORI KUBO ET AL.: "Gene-silencing potency of symmetric and asymmetric lipid-conjugated siRNAs and its correlation with Dicer recognition", BIOCONJUGATE CHEMISTRY, vol. 24, no. 12, 18 December 2013 (2013-12-18), pages 2045-2057, XP055347880, ISSN: 1043-1802, DOI: 10.1021/bc400391n figure 1 the whole document	1-45
A	SEUL-GI JO ET AL.: "Selection and optimization of asymmetric siRNA targeting the human c-MET gene", MOLECULES AND CELLS, vol. 32, no. 6, 1 December 2011 (2011-12-01), pages 543-548, XP055067178, ISSN: 1016-8478, DOI: 10.1007/s10059-011-0160-1 figures 1, 2 the whole document	1-45
A	SUN WOO HONG ET AL.: "Effect of the guide strand 3'-end structure on the gene-silencing potency of asymmetric siRNA", BIOCHEMICAL JOURNAL, vol. 461, no. 3, 1 August 2014 (2014-08-01), pages 427-434, XP055347879, GB ISSN: 0264-6021, DOI: 10.1042/BJ20140407 figure 2 the whole document	1-45
A	WO 2007/038788 A2 (THE CLEVELAND CLINIC FOUNDATION [US]; MARQUES J.T.; WILLIAMS B.R.G.) 5 April 2007 (2007-04-05) page 27; sequences 23, 24 the whole document	1-45
A	CHAN IL CHANG ET AL.: "Asymmetric shorter-duplex siRNA structures trigger efficient gene silencing with reduced nonspecific effects", MOLECULAR THERAPY, NATURE PUBLISHING GROUP, GB, vol. 17, no. 4, 1 April 2009 (2009-04-01), pages 725-732, XP002635991, ISSN: 1525-0024, DOI: 10.1038/MT.2008.298 [retrieved on 2009-01-20] the whole document	1-45
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/064951

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHANG CHANIL ET AL.: "The design, preparation, and evaluation of asymmetric small interfering RNA for specific gene silencing in mammalian cells", METHODS IN MOLECULAR BIOLOGY (CLIFTON, N.J.) 2013, vol. 942, 2013, pages 135-152, XP008183454, ISSN: 1940-6029 the whole document -----	1-45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/064951

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-45(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-45(partially)

An RNA molecule comprising an antisense strand comprising 5'-terminal and 3'-terminal nucleotides that are 17, 18, 19, 20, or 21 nucleotides apart, and a sense strand comprising a 5'-terminal nucleotide that is complementary to a nucleotide of the antisense strand other than its 3'-terminal nucleotide and a 3'-terminal nucleotide that is complementary to a nucleotide of the antisense strand, wherein at least 14 nucleotides of the antisense strand are complementary with the sense strand, and wherein at least 14 nucleotides of the antisense strand are colinear with the corresponding complementary nucleotides in the target nucleotide SEQ ID NO. 140. Products and methods related thereto.

2-25. claims: 1-45(partially)

As invention 1 but concerning SEQ ID NO:141-164.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/064951

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005007855	A2	27-01-2005	NONE
WO 2009029688	A2	05-03-2009	CA 2735166 A1 05-03-2009
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		WO 2009029690 A1 05-03-2009	
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			WO 2007038788 A2 05-04-2007