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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 September 2016 (01.09.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/138278 A2

(51) International Patent Classification:
C12N 15/11 (2006.01) *A61K 31/7105* (2006.01)

(21) International Application Number:
PCT/US2016/019584

(22) International Filing Date:
25 February 2016 (25.02.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/126,368 27 February 2015 (27.02.2015) US

(71) Applicant: **IDERA PHARMACEUTICALS, INC.** [US/US]; 167 Sidney Street, Cambridge, MA 02139 (US).

(72) Inventors: **AGRAWAL, Sudhir**; 61 Lamplighter Drive, Shrewsbury, MA 01545 (US). **JIANG, Wayne**; 15 Lyman Terrace, Walham, MA 02452 (US). **WANG, Daqing**; 7 Selfridge Road, Bedford, MA 01730 (US). **SEITZER, Jessica**; 32 Morrison Rd., Windham, NH 03087 (US). **ZHU, Fu-Gang**; 16 Woodland Road, Bedford, MA 01730 (US). **MAO, Xianzhi**; 2101 Seneca Run, Ambler, PA 09902 (US).

(74) Agents: **ZUCCHERO, Joseph, C.** et al.; Elmore Patent Law Group, P.C., 484 Groton Rd., Westford, MA 01886 (US).

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

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

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2016/138278 A2

(54) **Title**: COMPOSITIONS FOR INHIBITING CHECKPOINT GENE EXPRESSION AND USES THEREOF

(57) **Abstract**: The present invention is directed to compounds, compositions, and methods useful for modulating PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, ID02, CEACAM1, OX40, and/or OX40L mRNA or protein expression using gene silencing compounds comprising two or more single stranded antisense oligonucleotides that are linked through their 5 '-ends to allow the presence of two or more accessible 3 '-ends.

COMPOSITIONS FOR INHIBITING CHECKPOINT GENE EXPRESSION AND USES THEREOF

BACKGROUND OF THE INVENTION

Related Applications

[0001] This application claims the benefit of U.S. provisional patent application serial number 62/126,368, filed on February 27, 2015, the contents of which are incorporated herein by reference in its entirety.

Field of the invention

[0002] The present invention relates to compounds, compositions, and methods of use for the inhibition of checkpoint gene expression or for diagnosing, treating and/or preventing diseases and/or conditions that respond to the inhibition of checkpoint gene expression.

Summary of the related art

[0003] The immune system is a hosts defense against foreign antigens; however, in order to function properly a variety of checks and balances are required to protect against self-antigens (i.e., autoimmunity) and, at the same time, provide an appropriate response against foreign. Immune-activating and immune-suppressive receptors and ligands provide these regulatory checks and balances (see Pardoll et al., The blockade of immune checkpoints in cancer immunotherapy, Nat. Rev. Canc. 12, 252 (2012)).

[0004] Immune checkpoints refer to a group of endogenous immune-suppressive ligands and receptors that are crucial for the maintenance of self-tolerance and the protection of tissues from damage when the immune system is responding to an infection. (see Y.L. Wu, et al., Immunotherapies: The Blockade of Inhibitory Signals, Int. J. Biol. Sci. 8, 1420 (2012)) In response to the induction of an immune response expression of checkpoints increases. These checkpoints act as regulatory feedback to maintain immune homeostasis.

[0005] In patients with cancer, tumor mutations give rise to tumor-specific antigens that can be recognized by the immune system, particularly T-cells, leading to elimination of cancer cells. However, to defend themselves, tumor cells can co-opt immune checkpoint pathways to suppress the immune response in the tumor microenvironment and evade the host immune system by inhibiting T cells that might otherwise attack the tumor cells. (see J.F. Grosso & M.N. Jure-Kunkel; CTLA-4 blockade in tumor models: an overview of preclinical and translational research, Cancer Immun. 13, 5 (2013); M.E. Turnis, et al.;

Combinatorial immunotherapy: PD-1 may not be LAG-ing behind any more, OncoImmunology 1, 1172 (2012)).

[0006] Many previous cancer immunotherapies have likely been limited by these suppressive mechanisms. Thus there is a need to over these immunosuppressive mechanisms in order to enhance antitumor immunotherapy applications.

BRIEF SUMMARY OF THE INVENTION

[0007] The present invention is directed to compounds, compositions, and methods useful for modulating PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L mRNA or protein expression using gene silencing compounds comprising two or more single stranded antisense oligonucleotides that are linked through their 5'-ends to allow the presence of two or more accessible 3'-ends. The gene silencing compounds according to the invention effectively inhibit or decrease PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L mRNA or protein expression.

[0008] Provided herein are methods, compounds, and compositions for modulating expression of PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L mRNA and protein. In certain embodiments, compounds useful for modulating expression of PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L mRNA and protein are gene silencing compounds.

[0009] In certain embodiments, modulation can occur in a cell or tissue. In certain embodiments the cell is a tumor cell. In certain embodiments, the tissue is a tumor. In certain embodiments, the cell or tissue is in an animal. In certain embodiments, the animal is a human. In certain embodiments, PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L mRNA levels are reduced. In certain embodiments, PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L protein levels are reduced. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

[0010] Also provided are methods, compounds, and compositions useful for preventing, treating, and ameliorating diseases, disorders, and conditions. In certain embodiments, such diseases, disorders, and conditions are hyperproliferative diseases, disorders, and conditions. In certain embodiments such hyperproliferative diseases, disorders, and conditions include cancer as well as associated malignancies and metastases.

[0011] In certain embodiments, methods of treatment include administering a PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L gene

silencing compound or composition to an individual in need thereof. In certain embodiments, the gene silencing compound or composition is administered intratumorally.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0012] The invention relates to the therapeutic and prophylactic use of gene silencing compounds, also referred to as 3rd generation antisense (3GA) compounds, to down-regulate checkpoint mRNA or protein expression. Such molecules are useful, for example, in providing compositions for modulation of checkpoint gene expression or for treating and/or preventing diseases and/or conditions that are capable of responding to modulation of checkpoint gene expression in patients, subjects, animals or organisms.

[0013] The objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which the following terms have the ascribed meaning. Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis. Where permitted, all patents, applications, published applications and other publications, GENBANK Accession Numbers and associated sequence information obtainable through databases such as National Center for Biotechnology Information (NCBI) and other data referred to throughout in the disclosure herein are incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

[0014] The term "2'-O-substituted" means substitution of the 2' position of the pentose moiety with an –O- lower alkyl group containing 1-6 saturated or unsaturated carbon atoms (for example, but not limited to, 2'-O-methyl), or with an –O-aryl or allyl group having 2-6 carbon atoms, wherein such alkyl, aryl or allyl group may be unsubstituted or may be substituted, (for example, with 2'-O-methoxyethyl, ethoxy, methoxy, halo, hydroxyl, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carbalkoxyl, or amino groups); or with a hydroxyl, an amino or a halo group, but not with a 2'-H group. In some embodiments the oligonucleotides of the invention include four or five 2'-O-alky nucleotides at their 5' terminus, and/or four or five 2'-O-alky nucleotides at their 3' terminus.

[0015] The term "3'", when used directionally, generally refers to a region or position in a polynucleotide or oligonucleotide 3' (toward the 3'end of the nucleotide) from another region or position in the same polynucleotide or oligonucleotide.

[0016] The term "3' end" generally refers to the 3' terminal nucleotide of the component oligonucleotides. "Two or more oligonucleotides linked at their 3' ends" generally refers to a linkage between the 3' terminal nucleotides of the oligonucleotides which may be directly via 5', 3' or 2' hydroxyl groups, or indirectly, via a non-nucleotide linker. Such linkages may also be via a nucleoside, utilizing both 2' and 3' hydroxyl positions of the nucleoside. Such linkages may also utilize a functionalized sugar or nucleobase of a 3'terminal nucleotide.

[0017] The term "5'", when used directionally, generally refers to a region or position in a polynucleotide or oligonucleotide 5' (toward the 5'end of the nucleotide) from another region or position in the same polynucleotide or oligonucleotide.

[0018] The term "5' end" generally refers to the 5' terminal nucleotide of the component oligonucleotides. "Two or more single-stranded antisense oligonucleotides linked at their 5' ends" generally refers to a linkage between the 5' terminal nucleotides of the oligonucleotides which may be directly via 5', 3' or 2' hydroxyl groups, or indirectly, via a non-nucleotide linker. Such linkages may also be via a nucleoside, utilizing both 2' and 3' hydroxyl positions of the nucleoside. Such linkages may also utilize a functionalized sugar or nucleobase of a 5'terminal nucleotide.

[0019] The term "about" generally means that the exact number is not critical. Thus, oligonucleotides having one or two fewer nucleoside residues, or from one to several additional nucleoside residues are contemplated as equivalents of each of the embodiments described above.

[0020] The term "accessible" generally means when related to a compound according to the invention, that the relevant portion of the molecule is able to be recognized by the cellular components necessary to elicit an intended response to the compound.

[0021] The term "agonist" generally refers to a substance that binds to a receptor of a cell and induces a response. An agonist can be a naturally occurring substance such as bacterial DNA or a synthetic composition. A synthetic agonist often mimics the action of a naturally occurring substance such as a ligand.

[0022] The term "antigen" generally refers to a substance that is recognized and selectively bound by an antibody or by a T cell antigen receptor. Antigens may include but are not limited to peptides, proteins, lipids, carbohydrates, nucleosides, nucleotides, nucleic acids, and combinations thereof. Antigens may be natural or synthetic and generally induce an immune response that is specific for that antigen.

[0023] "Antisense activity" means any detectable or measurable activity attributable to the hybridization of a gene silencing compound to its target nucleic acid. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid.

[0024] As used herein, "Gene silencing oligonucleotide (GSO)", "Gene silencing compound", or "3rd generation antisense (3GA)" compound are used interchangeably to refer to an oligomeric compound comprising two or more single stranded antisense oligonucleotides that are linked through their 5'-ends to allow the presence of two or more accessible 3'-ends. Gene silencing compounds are capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

[0025] "Antisense inhibition" means reduction of target nucleic acid levels or target protein levels in the presence of a gene silencing compound complementary to a target nucleic acid as compared to target nucleic acid levels or target protein levels in the absence of the gene silencing compound.

[0026] "Antisense oligonucleotide" means a single-stranded oligonucleotide having a nucleobase sequence that permits hybridization to a corresponding region or segment of a target nucleic acid.

[0027] The term "biologic instability" generally refers to a molecule's ability to be degraded and subsequently inactivated *in vivo*. For oligonucleotides, such degradation results from exonuclease activity and/or endonuclease activity, wherein exonuclease activity refers to cleaving nucleotides from the 3' or 5' end of an oligonucleotide, and endonuclease activity refers to cleaving phosphodiester bonds at positions other than at the ends of the oligonucleotide.

[0028] The term "cancer" generally refers to, without limitation, any malignant growth or tumor caused by abnormal or uncontrolled cell proliferation and/or division. Cancers may occur in humans and/or mammals and may arise in any and all tissues. Treating a patient having cancer may include administration of a compound, pharmaceutical formulation or vaccine according to the invention such that the abnormal or uncontrolled cell proliferation and/or division, or metastasis is affected.

[0029] The term "carrier" generally encompasses any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microspheres, liposomal encapsulation, or other material for use in pharmaceutical formulations. It will be understood that the characteristics of the carrier, excipient or diluent will depend on the route of administration for a particular application. The preparation of pharmaceutically acceptable

formulations containing these materials is described in, for example, *Remington's Pharmaceutical Sciences*, 18th Edition, ed. A. Gennaro, Mack Publishing Co., Easton, PA, 1990.

[0030] The term "co-administration" or "co-administered" generally refers to the administration of at least two different substances. Co-administration refers to simultaneous administration, as well as temporally spaced order of up to several days apart, of at least two different substances in any order, either in a single dose or separate doses.

[0031] The term "in combination with" generally means administering two or more agents (e.g., a gene silencing compound according to the invention and another agent) such that there is an overlap of an effect of each agent on the patient. Such administration may be done in any order, including simultaneous administration, as well as temporally spaced order from a few seconds up to several days apart. In some embodiments, the administration of the agents are spaced sufficiently close together such that a combinatorial effect is achieved. Such combination treatment may also include more than a single administration of the compound according to the invention and/or independently the other agent. The administration of the compound according to the invention and the other agent may be by the same or different routes. In some embodiments, administration of at least one agent is made while the other agent is still present at a therapeutic level in the subject.

[0032] The term "complementary" is intended to mean the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

[0033] "Contiguous nucleobases" means nucleobases immediately adjacent to each other.

[0034] The term "individual" or "subject" or "patient" generally refers to a mammal, such as a human.

[0035] "CEACAM1 nucleic acid" means any nucleic acid encoding CEACAM1. For example, in certain embodiments, a CEACAM1 nucleic acid includes a DNA sequence encoding CEACAM1, an RNA sequence transcribed from DNA encoding CEACAM1 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding CEACAM1. "CEACAM1 mRNA" means an mRNA encoding a CEACAM1 protein.

[0036] "CTLA4 nucleic acid" means any nucleic acid encoding CTLA4. For example, in certain embodiments, a CTLA4 nucleic acid includes a DNA sequence encoding CTLA4, an RNA sequence transcribed from DNA encoding CTLA4 (including genomic DNA

comprising introns and exons), and an mRNA sequence encoding CTLA4. "CTLA4 mRNA" means an mRNA encoding a CTLA4 protein.

[0037] "Fully complementary" or "100% complementary" means each nucleobase of a first nucleic acid has a complementary nucleobase in a second nucleic acid. In certain embodiments, a first nucleic acid is an antisense compound and a target nucleic acid is a second nucleic acid.

[0038] "Hybridization" means the annealing of complementary nucleic acid molecules. In certain embodiments, complementary nucleic acid molecules include an antisense compound and a target nucleic acid.

[0039] "IDO1 nucleic acid" means any nucleic acid encoding IDO1. For example, in certain embodiments, a IDO1 nucleic acid includes a DNA sequence encoding IDO1, an RNA sequence transcribed from DNA encoding IDO1 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding IDO1. "IDO1 mRNA" means an mRNA encoding an IDO1 protein.

[0040] "IDO2 nucleic acid" means any nucleic acid encoding IDO2. For example, in certain embodiments, a IDO2 nucleic acid includes a DNA sequence encoding IDO2, an RNA sequence transcribed from DNA encoding IDO2 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding IDO2. "IDO2 mRNA" means an mRNA encoding an IDO2 protein.

[0041] "Inhibiting PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L mRNA or protein expression" means reducing expression of PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L mRNA and/or protein levels in the presence of a gene silencing compound according to the invention as compared to expression of PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L mRNA and/or protein levels in the absence of a gene silencing compound according to the invention.

[0042] The term “kinase inhibitor” generally refers to molecules that antagonize or inhibit phosphorylation-dependent cell signaling and/or growth pathways in a cell. Kinase inhibitors may be naturally occurring or synthetic and include small molecules that have the potential to be administered as oral therapeutics. Kinase inhibitors have the ability to rapidly and specifically inhibit the activation of the target kinase molecules. Protein kinases are attractive drug targets, in part because they regulate a wide variety of signaling and growth pathways and include many different proteins. As such, they have great potential in the treatment of diseases involving kinase signaling, including cancer, cardiovascular disease,

inflammatory disorders, diabetes, macular degeneration and neurological disorders. A non-limiting example of a kinase inhibitor is sorafenib.

[0043] "LAG3 nucleic acid" means any nucleic acid encoding LAG3. For example, in certain embodiments, a LAG3 nucleic acid includes a DNA sequence encoding LAG3, an RNA sequence transcribed from DNA encoding LAG3 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding LAG3. "LAG3 mRNA" means an mRNA encoding a LAG3 protein.

[0044] The term "linear synthesis" generally refers to a synthesis that starts at one end of an oligonucleotide and progresses linearly to the other end. Linear synthesis permits incorporation of either identical or non-identical (in terms of length, base composition and/or chemical modifications incorporated) monomeric units into an oligonucleotide.

[0045] The term "mammal" is expressly intended to include warm blooded, vertebrate animals, including, without limitation, humans, non-human primates, rats, mice, cats, dogs, horses, cattle, cows, pigs, sheep and rabbits.

[0046] The term "nucleoside" generally refers to compounds consisting of a sugar, usually ribose, deoxyribose, pentose, arabinose or hexose, and a purine or pyrimidine base.

[0047] The term "nucleotide" generally refers to a nucleoside comprising a phosphorous-containing group attached to the sugar.

[0048] The term "modified nucleoside" or "nucleotide derivative" generally is a nucleoside that includes a modified heterocyclic base, a modified sugar moiety, or any combination thereof. In some embodiments, the modified nucleoside or nucleotide derivative is a non-natural pyrimidine or purine nucleoside, as herein described. For purposes of the invention, a modified nucleoside or nucleotide derivative, a pyrimidine or purine analog or non-naturally occurring pyrimidine or purine can be used interchangeably and refers to a nucleoside that includes a non-naturally occurring base and/or non-naturally occurring sugar moiety. For purposes of the invention, a base is considered to be non-natural if it is not guanine, cytosine, adenine, thymine or uracil and a sugar is considered to be non-natural if it is not β-ribo-furanoside or 2'-deoxyribo-furanoside.

[0049] The term "modified oligonucleotide" as used herein describes an oligonucleotide in which at least two of its nucleotides are covalently linked via a synthetic linkage, i.e., a linkage other than a phosphodiester linkage between the 5' end of one nucleotide and the 3' end of another nucleotide in which the 5' nucleotide phosphate has been replaced with any number of chemical groups. The term "modified oligonucleotide" also

encompasses 2'-O,4'-C-methylene-b-D-ribofuranosyl nucleic acids, arabinose nucleic acids, substituted arabinose nucleic acids, hexose nucleic acids, peptide nucleic acids, morpholino, and oligonucleotides having at least one nucleotide with a modified base and/or sugar, such as a 2'-O-substituted, a 5- methylcytosine and/or a 3'-O-substituted ribonucleotide.

[0050] The term "nucleic acid" encompasses a genomic region or an RNA molecule transcribed therefrom. In some embodiments, the nucleic acid is mRNA.

[0051] The term "linker" generally refers to any moiety that can be attached to an oligonucleotide by way of covalent or non-covalent bonding through a sugar, a base, or the backbone. The non-covalent linkage may be, without limitation, electrostatic interactions, hydrophobic interactions, π-stacking interactions, hydrogen bonding and combinations thereof. Non-limiting examples of such non-covalent linkage includes Watson-Crick base pairing, Hoogsteen base pairing, and base stacking. The linker can be used to attach two or more nucleosides or can be attached to the 5' and/or 3' terminal nucleotide in the oligonucleotide. Such linker can be either a non-nucleotide linker or a nucleoside linker.

[0052] The term "non-nucleotide linker" generally refers to a chemical moiety, other than a linkage directly between two nucleotides that can be attached to an oligonucleotide by way of covalent or non-covalent bonding. Preferably such non-nucleotide linker is from about 2 angstroms to about 200 angstroms in length, and may be either in a cis or trans orientation.

[0053] The term "internucleotide linkage" generally refer to a chemical linkage to join two nucleosides through their sugars (e.g. 3'-3', 2'-3', 2'-5', 3'-5', 5'-5') consisting of a phosphorous atom and a charged, or neutral group (e.g., phosphodiester, phosphorothioate, phosphorodithioate or methylphosphonate) between adjacent nucleosides.

[0054] The term "oligonucleotide" refers to a polynucleoside formed from a plurality of linked nucleoside units, which may include, for example, deoxyribonucleotides or ribonucleotides, synthetic or natural nucleotides, phosphodiester or modified linkages, natural bases or modified bases natural sugars or modified sugars, or combinations of these components. The nucleoside units may be part of viruses, bacteria, cell debris or oligonucleotide-based compositions (for example, siRNA and microRNA). Such oligonucleotides can also be obtained from existing nucleic acid sources, including genomic or cDNA, but are preferably produced by synthetic methods. In certain embodiments each nucleoside unit includes a heterocyclic base and a pentofuranosyl, trehalose, arabinose, 2'-deoxy-2'-substituted nucleoside, 2'-deoxy-2'-substituted arabinose, 2'-O-

substitutedarabinose or hexose sugar group. The nucleoside residues can be coupled to each other by any of the numerous known internucleoside linkages. Such internucleoside linkages include, without limitation, phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, alkylphosphonate, alkylphosphonothioate, phosphotriester, phosphoramidate, siloxane, carbonate, carboalkoxy, acetamidate, carbamate, morpholino, borano, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphorothioate, and sulfone internucleoside linkages. The term "oligonucleotide" also encompasses polynucleosides having one or more stereospecific internucleoside linkage (e.g., (R_P)- or (S_P)-phosphorothioate, alkylphosphonate, or phosphotriester linkages). As used herein, the terms "oligonucleotide" and "dinucleotide" are expressly intended to include polynucleosides and dinucleosides having any such internucleoside linkage, whether or not the linkage comprises a phosphate group. In certain exemplary embodiments, these internucleoside linkages may be phosphodiester, phosphorothioate or phosphorodithioate linkages, or combinations thereof. In exemplary embodiments, the nucleotides of the synthetic oligonucleotides are linked by at least one phosphorothioate internucleotide linkage. The phosphorothioate linkages may be mixed Rp and Sp enantiomers, or they may be stereoregular or substantially stereoregular in either Rp or Sp form (see Iyer et al. (1995) Tetrahedron Asymmetry 6:1051-1054). In certain embodiments, one or more of the oligonucleotides within the antisense compositions of the invention contain one or more 2'-O,4'-C-methylene-b-D-ribofuranosyl nucleic acids, wherein the ribose is modified with a bond between the 2' and 4' carbons, which fixes the ribose in the 3'-endo structural conformation.

[0055] "OX40 nucleic acid" means any nucleic acid encoding OX40. For example, in certain embodiments, a OX40 nucleic acid includes a DNA sequence encoding OX40, an RNA sequence transcribed from DNA encoding OX40 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding OX40. "OX40 mRNA" means an mRNA encoding an OX40 protein.

[0056] "OX40L nucleic acid" means any nucleic acid encoding OX40L. For example, in certain embodiments, a OX40L nucleic acid includes a DNA sequence encoding OX40L, an RNA sequence transcribed from DNA encoding OX40L (including genomic DNA comprising introns and exons), and an mRNA sequence encoding OX40L. "OX40L mRNA" means an mRNA encoding an OX40L protein.

[0057] "PD1 nucleic acid" means any nucleic acid encoding PD1. For example, in certain embodiments, a PD1 nucleic acid includes a DNA sequence encoding PD1, an RNA

sequence transcribed from DNA encoding PD1 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding PD1. "PD1 mRNA" means an mRNA encoding a PD1 protein.

[0058] "PDL1 nucleic acid" means any nucleic acid encoding PDL1. For example, in certain embodiments, a PDL1 nucleic acid includes a DNA sequence encoding PDL1, an RNA sequence transcribed from DNA encoding PDL1 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding PDL1. "PDL1 mRNA" means an mRNA encoding a PDL1 protein.

[0059] The term "peptide" generally refers to oligomers or polymers of amino acids that are of sufficient length and composition to affect a biological response, for example, antibody production or cytokine activity whether or not the peptide is a hapten. The term "peptide" may include modified amino acids (whether or not naturally or non-naturally occurring), where such modifications include, but are not limited to, phosphorylation, glycosylation, pegylation, lipidization, and methylation.

[0060] The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of a compound according to the invention or the biological activity of a compound according to the invention.

[0061] The term "physiologically acceptable" refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. Preferably, the biological system is a living organism, such as a mammal, particularly a human.

[0062] The term "prophylactically effective amount" generally refers to an amount sufficient to prevent or reduce the development of an undesired biological effect.

[0063] "Portion" means a defined number of contiguous (i.e., linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an antisense compound.

[0064] "Single-stranded oligonucleotide" means an oligonucleotide which is not hybridized to a complementary strand.

[0065] "Specifically hybridizable" refers to a gene silencing compound having a sufficient degree of complementarity between an antisense oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays and therapeutic treatments.

[0066] "Targeting" or "targeted" means the process of design and selection of a gene silencing compound that will specifically hybridize to a target nucleic acid and induce a desired effect.

[0067] "Target nucleic acid," "target RNA," "target mRNA," and "target RNA transcript" all refer to a nucleic acid capable of being targeted by gene silencing compounds.

[0068] "Target segment" means the sequence of nucleotides of a target nucleic acid to which a gene silencing compound is targeted. "5' target site" refers to the 5'-most nucleotide of a target segment. "3' target site" refers to the 3'-most nucleotide of a target segment.

[0069] The term "therapeutically effective amount" or "pharmaceutically effective amount" generally refers to an amount sufficient to affect a desired biological effect, such as a beneficial result, including, without limitation, prevention, diminution, amelioration or elimination of signs or symptoms of a disease or disorder. Thus, the total amount of each active component of the pharmaceutical composition or method is sufficient to show a meaningful patient benefit, for example, but not limited to, healing of chronic conditions characterized by immune stimulation. Thus, a "pharmaceutically effective amount" will depend upon the context in which it is being administered. A pharmaceutically effective amount may be administered in one or more prophylactic or therapeutic administrations. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

[0070] "TIM3 nucleic acid" means any nucleic acid encoding TIM3. For example, in certain embodiments, a TIM3 nucleic acid includes a DNA sequence encoding TIM3, an RNA sequence transcribed from DNA encoding TIM3 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding TIM3. "TIM3 mRNA" means an mRNA encoding a TIM3 protein.

[0071] The term "treatment" generally refers to an approach intended to obtain a beneficial or desired result, which may include alleviation of symptoms, or delaying or ameliorating a disease progression.

[0072] The term "gene expression" generally refers to process by which information from a gene is used in the synthesis of a functional gene product, which may be a protein. The process may involve transcription, RNA splicing, translation, and post-translational modification of a protein, and may include mRNA, preRNA, ribosomal RNA, and other templates for protein synthesis.

[0073] In certain embodiments provided are methods, compounds, and compositions for inhibiting PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L mRNA or protein expression. In certain embodiments the compounds are antisense oligonucleotides, double stranded or single-stranded siRNA compounds, or gene silencing compounds.

[0074] As used herein, gene silencing compounds according to the invention comprise two or more single-stranded antisense oligonucleotides linked at their 5' ends, wherein the compounds have two or more accessible 3' ends. The general structure of the oligonucleotide-based compounds of the invention may be described by the following formula I:

3'-Nn...N1N2N3N4-5'-L-5'-N8N7N6N5...Nm-3' (Formula I),

wherein L is a nucleotide linker or non-nucleotide linker; N1-N8, at each occurrence, is independently a nucleotide or nucleotide derivative; Nm and Nn, at each occurrence, are independently a nucleotide or nucleotide derivative; and wherein m and n are independently numbers from 0 to about 40.

[0075] The linkage at the 5' ends of the component oligonucleotides is independent of the other oligonucleotide linkages and may be directly via 5', 3' or 2' hydroxyl groups, or indirectly, via a non-nucleotide linker or a nucleoside, utilizing either the 2' or 3' hydroxyl positions of the nucleoside. Linkages may also utilize a functionalized sugar or nucleobase of a 5' terminal nucleotide.

[0076] In certain embodiments provided are gene silencing compounds targeted to a mouse or human PD1 nucleic acid. In certain embodiments, the mouse PD1 nucleic acid is the sequence set forth in GENBANK Accession No. NM_008798 (incorporated herein as SEQ ID NO: 387) or the human PD1 nucleic acid is the sequence set forth in GENBANK Accession No. NM_005018 (incorporated herein as SEQ ID NO: 388).

[0077] In certain embodiments provided are gene silencing compounds targeted to a mouse or human PDL1 nucleic acid. In certain embodiments, the mouse PDL1 nucleic acid is the sequence set forth in GENBANK Accession No. NM_021893 (incorporated herein as SEQ ID NO: 389) or the human PDL1 nucleic acid is the sequence set forth in GENBANK Accession No. NM_014143 (incorporated herein as SEQ ID NO: 390).

[0078] In certain embodiments provided are gene silencing compounds targeted to a mouse or human IDO1 nucleic acid. In certain embodiments, the mouse IDO1 nucleic acid is the sequence set forth in GENBANK Accession No. NM_008324 (incorporated herein as

SEQ ID NO: 391) or the human IDO1 nucleic acid is the sequence set forth in GENBANK Accession No. NM_002164 (incorporated herein as SEQ ID NO: 392).

[0079] In certain embodiments provided are gene silencing compounds targeted to a mouse or human LAG3 nucleic acid. In certain embodiments, the mouse LAG3 nucleic acid is the sequence set forth in GENBANK Accession No. NM_008479 (incorporated herein as SEQ ID NO: 393) or the human LAG3 nucleic acid is the sequence set forth in GENBANK Accession No. NM_002286 (incorporated herein as SEQ ID NO: 394).

[0080] In certain embodiments provided are gene silencing compounds targeted to a mouse or human TIM3 nucleic acid. In certain embodiments, the mouse TIM3 nucleic acid is the sequence set forth in GENBANK Accession No. NM_134250 (incorporated herein as SEQ ID NO: 395) or the human TIM3 nucleic acid is the sequence set forth in GENBANK Accession No. NM_032782 (incorporated herein as SEQ ID NO: 396).

[0081] In certain embodiments provided are gene silencing compounds targeted to a mouse or human CTLA4 nucleic acid. In certain embodiments, the mouse CTLA4 nucleic acid is the sequence set forth in GENBANK Accession No. NM_009843 (incorporated herein as SEQ ID NO: 397) or the human CTLA4 nucleic acid is the sequence set forth in GENBANK Accession No. NM_005214 (incorporated herein as SEQ ID NO: 398).

[0082] In certain embodiments provided are gene silencing compounds targeted to a mouse or human IDO2 nucleic acid. In certain embodiments, the mouse IDO2 nucleic acid is the sequence set forth in GENBANK Accession No. NM_145949 (incorporated herein as SEQ ID NO: 399) or the human IDO2 nucleic acid is the sequence set forth in GENBANK Accession No. NM_194294 (incorporated herein as SEQ ID NO: 400).

[0083] In certain embodiments provided are gene silencing compounds targeted to a mouse or human CEACAM1 nucleic acid. In certain embodiments, the mouse CEACAM1 nucleic acid is the sequence set forth in GENBANK Accession No. NM_001039187 (incorporated herein as SEQ ID NO: 401) or the human CEACAM1 nucleic acid is the sequence set forth in GENBANK Accession No. NM_001205344 (incorporated herein as SEQ ID NO: 402).

[0084] In certain embodiments provided are gene silencing compounds targeted to a mouse or human OX40 nucleic acid. In certain embodiments, the mouse OX40 nucleic acid is the sequence set forth in GENBANK Accession No. NM_011659 (incorporated herein as SEQ ID NO: 403) or the human OX40 nucleic acid is the sequence set forth in GENBANK Accession No. NM_003327 (incorporated herein as SEQ ID NO: 404).

[0085] In certain embodiments provided are gene silencing compounds targeted to a mouse or human OX40L nucleic acid. In certain embodiments, the mouse OX40L nucleic acid is the sequence set forth in GENBANK Accession No. NM_009452 (incorporated herein as SEQ ID NO: 405) or the human OX40L nucleic acid is the sequence set forth in GENBANK Accession No. NM_003326 (incorporated herein as SEQ ID NO: 406).

[0086] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389, SEQ ID NO: 390, SEQ ID NO: 391, SEQ ID NO: 392, SEQ ID NO: 393, SEQ ID NO: 394, SEQ ID NO: 395, SEQ ID NO: 396, SEQ ID NO: 397, SEQ ID NO: 398, SEQ ID NO: 399, SEQ ID NO: 400, SEQ ID NO: 401, SEQ ID NO: 402, SEQ ID NO: 403, SEQ ID NO: 404, SEQ ID NO: 405, or SEQ ID NO: 406. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389, SEQ ID NO: 390, SEQ ID NO: 391, SEQ ID NO: 392, SEQ ID NO: 393, SEQ ID NO: 394, SEQ ID NO: 395, SEQ ID NO: 396, SEQ ID NO: 397, SEQ ID NO: 398, SEQ ID NO: 399, SEQ ID NO: 400, SEQ ID NO: 401, SEQ ID NO: 402, SEQ ID NO: 403, SEQ ID NO: 404, SEQ ID NO: 405, or SEQ ID NO: 406. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389, SEQ ID NO: 390, SEQ ID NO: 391, SEQ ID NO: 392, SEQ ID NO: 393, SEQ ID NO: 394, SEQ ID NO: 395, SEQ ID NO: 396, SEQ ID NO: 397, SEQ ID NO: 398, SEQ ID NO: 399, SEQ ID NO: 400, SEQ ID NO: 401, SEQ ID NO: 402, SEQ ID NO: 403, SEQ ID NO: 404, SEQ ID NO: 405, or SEQ ID NO: 406. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389, SEQ ID NO: 390, SEQ ID NO: 391, SEQ ID NO: 392, SEQ ID NO: 393, SEQ ID NO: 394, SEQ ID NO: 395, SEQ ID NO: 396, SEQ ID NO: 397, SEQ ID NO: 398, SEQ ID NO: 399, SEQ ID NO: 400, SEQ ID NO: 401, SEQ ID NO: 402, SEQ ID NO: 403, SEQ ID NO: 404, SEQ ID NO: 405, or SEQ ID NO: 406.

[0087] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387, SEQ ID NO: 389, SEQ ID NO: 391, SEQ ID NO: 393, SEQ ID NO: 395, SEQ ID NO: 397, SEQ ID NO: 399, SEQ ID NO: 401, SEQ ID NO: 403, or SEQ ID NO: 405. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387, SEQ ID NO: 389, SEQ ID NO: 391, SEQ ID NO: 393, SEQ ID NO: 395, SEQ ID NO: 397, SEQ ID NO: 399, SEQ ID NO: 401, SEQ ID NO: 403, or SEQ ID NO: 405. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387, SEQ ID NO: 389, SEQ ID NO: 391, SEQ ID NO: 393, SEQ ID NO: 395, SEQ ID NO: 397, SEQ ID NO: 399, SEQ ID NO: 401, SEQ ID NO: 403, or SEQ ID NO: 405. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387, SEQ ID NO: 389, SEQ ID NO: 391, SEQ ID NO: 393, SEQ ID NO: 395, SEQ ID NO: 397, SEQ ID NO: 399, SEQ ID NO: 401, SEQ ID NO: 403, or SEQ ID NO: 405.

[0088] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 388, SEQ ID NO: 390, SEQ ID NO: 392, SEQ ID NO: 394, SEQ ID NO: 396, SEQ ID NO: 398, SEQ ID NO: 400, SEQ ID NO: 402, SEQ ID NO: 404, or SEQ ID NO: 406. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 388, SEQ ID NO: 390, SEQ ID NO: 392, SEQ ID NO: 394, SEQ ID NO: 396, SEQ ID NO: 398, SEQ ID NO: 400, SEQ ID NO: 402, SEQ ID NO: 404, or SEQ ID NO: 406. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of

SEQ ID NO: 388, SEQ ID NO: 390, SEQ ID NO: 392, SEQ ID NO: 394, SEQ ID NO: 396, SEQ ID NO: 398, SEQ ID NO: 400, SEQ ID NO: 402, SEQ ID NO: 404, or SEQ ID NO: 406. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 388, SEQ ID NO: 390, SEQ ID NO: 392, SEQ ID NO: 394, SEQ ID NO: 396, SEQ ID NO: 398, SEQ ID NO: 400, SEQ ID NO: 402, SEQ ID NO: 404, or SEQ ID NO: 406.

[0089] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387.

[0090] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 388. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 388. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 388. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at

least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 388.

[0091] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 389. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 389. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 389. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 389.

[0092] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 390. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 390. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 390. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 390.

[0093] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 391. Certain embodiments provide compounds

comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 391. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 391. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 391.

[0094] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 392. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 392. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 392. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 392.

[0095] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 393. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 393. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 393. In certain embodiments, the

two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 393.

[0096] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 394. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 394. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 394. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 394.

[0097] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 395. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 395. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 395. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 395.

[0098] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase

sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 396. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 396. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 396. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 396.

[0099] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 397. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 397. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 397. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 397.

[00100] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 398. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 398. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides

having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 398. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 398.

[00101] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 399. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 399. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 399. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 399.

[00102] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 400. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 400. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 400. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 400.

[00103] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 401. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 401. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 401. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 401.

[00104] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 402. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 402. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 402. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 402.

[00105] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 403. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases

complementary to an equal length portion of SEQ ID NO: 403. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 403. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 403.

[00106] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 404. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 404. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 404. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 404.

[00107] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 405. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 405. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 405. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at

least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 405.

[00108] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 406. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 406. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 406. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 406.

[00109] In certain embodiments, the nucleobase sequence of the oligonucleotides of the gene silencing compound are, independently, at least 90% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, or 406. In certain embodiments, the nucleobase sequence of the oligonucleotides of the gene silencing compound are, independently, at least 95% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, or 406. In certain embodiments, the oligonucleotides of the gene silencing compound are at least 99% complementary over its entire length to SEQ ID NO: 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, or 406. In certain embodiments, the nucleobase sequence of the oligonucleotides of the gene silencing compound are 100% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, or 406.

[00110] In certain embodiments, the oligonucleotides of the gene silencing compound are, independently, 12 to 30 nucleotides in length. In other words, the oligonucleotides are from 12 to 30 linked nucleobases. In other embodiments, the oligonucleotides, independently, consist of 15 to 28, 18 to 24, 19 to 22, or 20 linked nucleobases. In certain

such embodiments, the oligonucleotides, independently, consist of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 linked nucleobases in length, or a range defined by any two of the above values.

[00111] In certain embodiments, a target region is a structurally defined region of the target nucleic acid. For example, a target region may encompass a 3′ UTR, a 5′ UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L can be obtained by accession number from sequence databases such as NCBI and such information is incorporated herein by reference. In certain embodiments, a target region may encompass the sequence from a 5′ target site of one target segment within the target region to a 3′ target site of another target segment within the same target region.

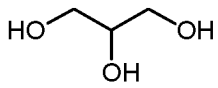
[00112] Certain embodiments provide a composition comprising a 3GA compound as described herein, or a salt thereof, and a pharmaceutically acceptable carrier or diluent. Certain embodiments provide a composition comprising two or more 3GA compounds as described herein, or a salt thereof, and a pharmaceutically acceptable carrier or diluent. The two or more 3GA compounds can inhibit the mRNA or protein expression of the same target or can inhibit the mRNA or protein expression of different targets.

[00113] In certain embodiments, the 3GA compounds according to the invention comprise two identical or different sequences linked at their 5'–5' ends via a phosphodiester, phosphorothioate or non-nucleoside linker. 3GA compounds according to the invention that comprise identical sequences are able to bind to a specific mRNA via Watson-Crick hydrogen bonding interactions and inhibit mRNA and protein expression. Gene silencing compounds according to the invention that comprise different sequences are able to bind to two or more different regions of one or more mRNA target and inhibit mRNA and protein expression. Such compounds are comprised of heteronucleotide sequences complementary to target mRNA and form stable duplex structures through Watson-Crick hydrogen bonding.

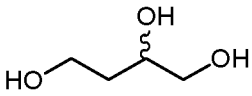
[00114] In certain embodiments, gene silencing compounds according to the invention are useful in treating and/or preventing diseases wherein inhibiting PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L expression would be beneficial. Gene silencing compounds according to the invention include, but are not limited to, antisense oligonucleotides comprising naturally occurring nucleotides, modified nucleotides, modified oligonucleotides and/or backbone modified oligonucleotides.

[00115] The oligonucleotides of the 3GA compounds are linked through their 5'-ends to allow the presence of two or more accessible 3'-ends. In certain embodiments, the oligonucleotides are linked through one or more of the non-nucleotide linkers listed in Table 1. In certain embodiments, a single linker listed in Table 1 is used to link the oligonucleotides of the gene silencing compounds. In certain embodiments, the linker is small molecule linker such as glycerol or a glycerol homolog of the formula $HO\text{-}(CH_2)_o\text{-}CH(OH)\text{-}(CH_2)_p\text{-}OH$, wherein o and p independently are integers from 1 to about 6, from 1 to about 4 or from 1 to about 3. In some other embodiments, the small molecule linker is a derivative of 1,3-diamino-2-hydroxypropane. Some such derivatives have the formula $HO\text{-}(CH_2)_m\text{-}C(O)NH\text{-}CH_2\text{-}CH(OH)\text{-}CH_2\text{-}NHC(O)\text{-}(CH_2)_m\text{-}OH$, wherein m is an integer from 0 to about 10, from 0 to about 6, from 2 to about 6 or from 2 to about 4. Representative non-nucleotide linkers are set forth in Table 1.

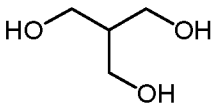
Table 1: Representative Non-Nucleotide Linkers



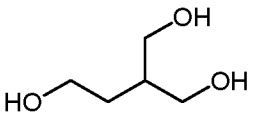
Glycerol (1,2,3-Propanetriol)



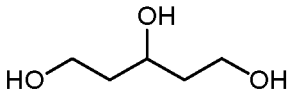
1,2,4-Butanetriol



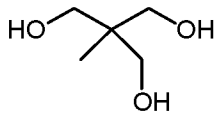
2-(hydroxymethyl)-1,3-propanediol



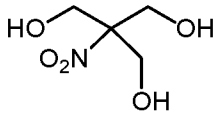
2-(hydroxymethyl)1,4-butanediol



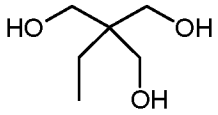
1,3,5-Pentanetriol



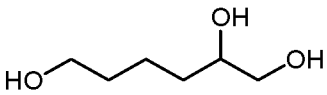
1,1,1-Tris(hydroxymethyl)ethane



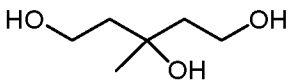
1,1,1-Tris(hydroxymethyl)nitromethane



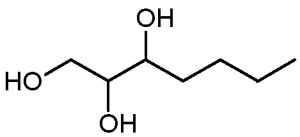
1,1,1-Tris(hydroxymethyl)propane



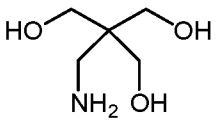
1,2,6-Hexanetriol



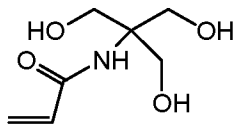
3-Methyl-1,3,5-pentanetriol



1,2,3-Heptanetriol

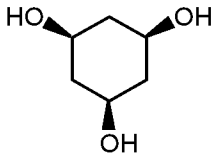


2-Amino-2-(hydroxymethyl)-1,3-propanediol

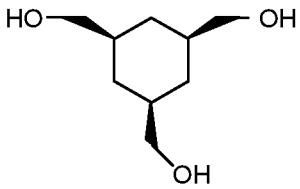


N-[Tris(hydroxymethyl)methyl]acrylamide

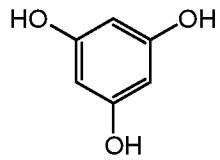
Table 1: Continued



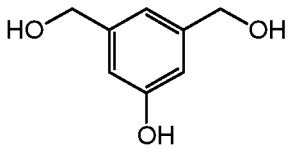
cis-1,3,5-Cyclohexanetriol



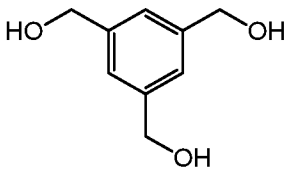
cis-1,3,5-Tri(hydroxymethyl)cyclohexane



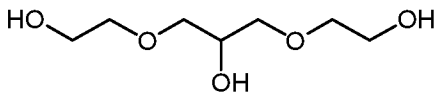
1,3,5,-Trihydroxyl-benzene



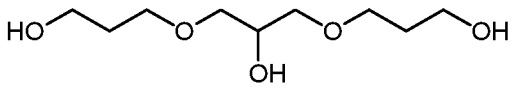
3,5,-Di(hydroxymethyl)phenol



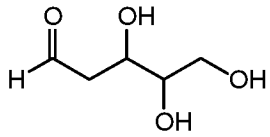
1,3,5,-Tri(hydroxymethyl)benzene



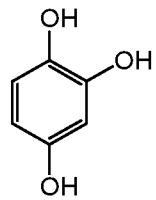
1,3-Di(hydroxyethoxy)-2-hydroxyl-propane



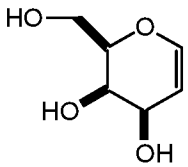
1,3-Di(hydroxypropoxy)-2-hydroxyl-propane



2-Deoxy-D-ribose

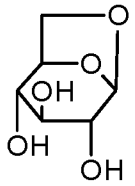


1,2,4,-Trihydroxyl-benzene

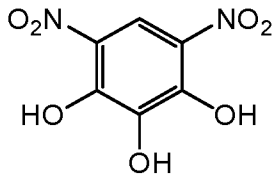


D-Galactoal

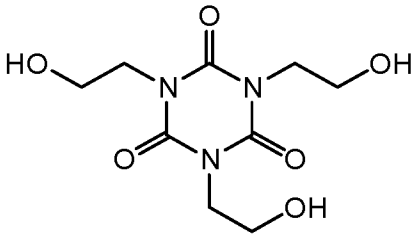
Table 1: Continued



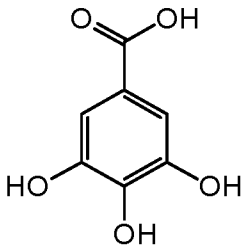
1,6-anhydro-β-D-Glucose



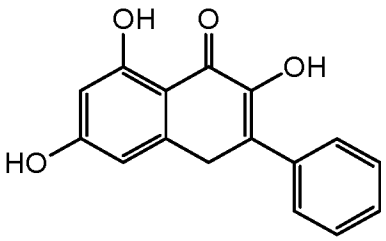
4,6-Nitropyrogallol



1,3,5-Tris(2-hydroxyethyl)-Cyanuric acid

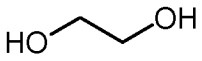


Gallic acid



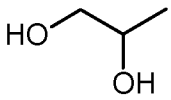
3,5,7-Trihydroxyflavone

Table 1: Continued

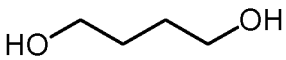


Ethylene glycol

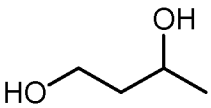
1,3-Propanediol



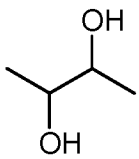
1,2-Propanediol



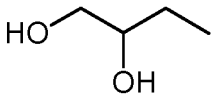
1,4-Butanediol



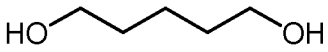
1,3-Butanediol



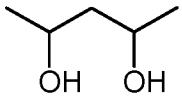
2,3-Butanediol



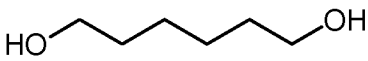
1,4-Butanediol



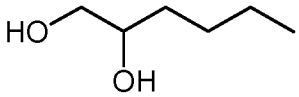
1,5-Pentanediol



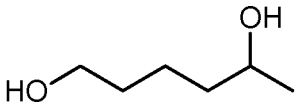
2,4-Pentanediol



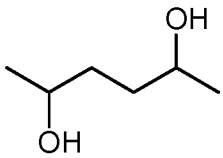
1,6-Hexanediol



1,2-Hexanediol



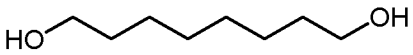
1,5-Hexanediol



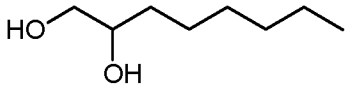
2,5-Hexanediol

Table 1: Continued

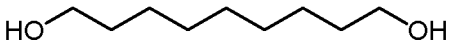
1,7-Heptanediol



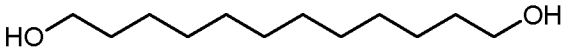
1,8-Octanediol



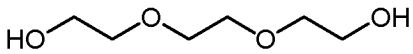
1,2-Octanediol



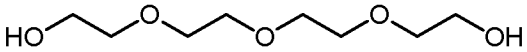
1,9-Nonanediol



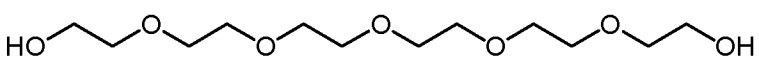
1,12-Dodecanediol



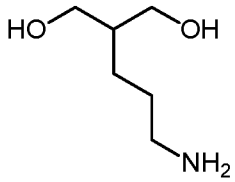
Triethylene glycol



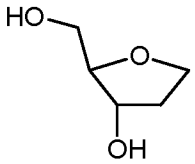
Tetraethylene glycol



Hexaethylene glycol



2-(1-Aminopropyl)-1,3-propanediol



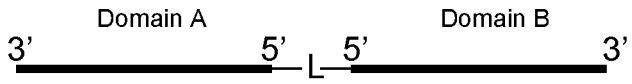
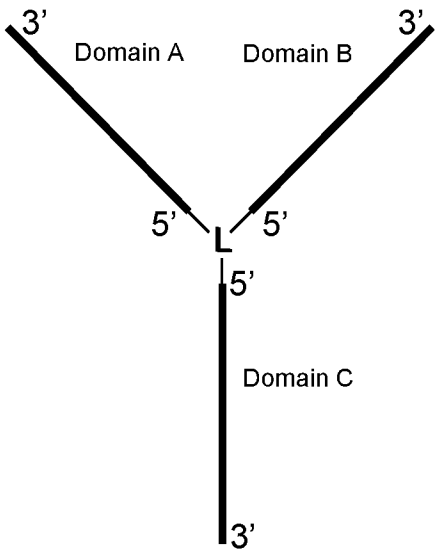
1,2-Dideoxyribose

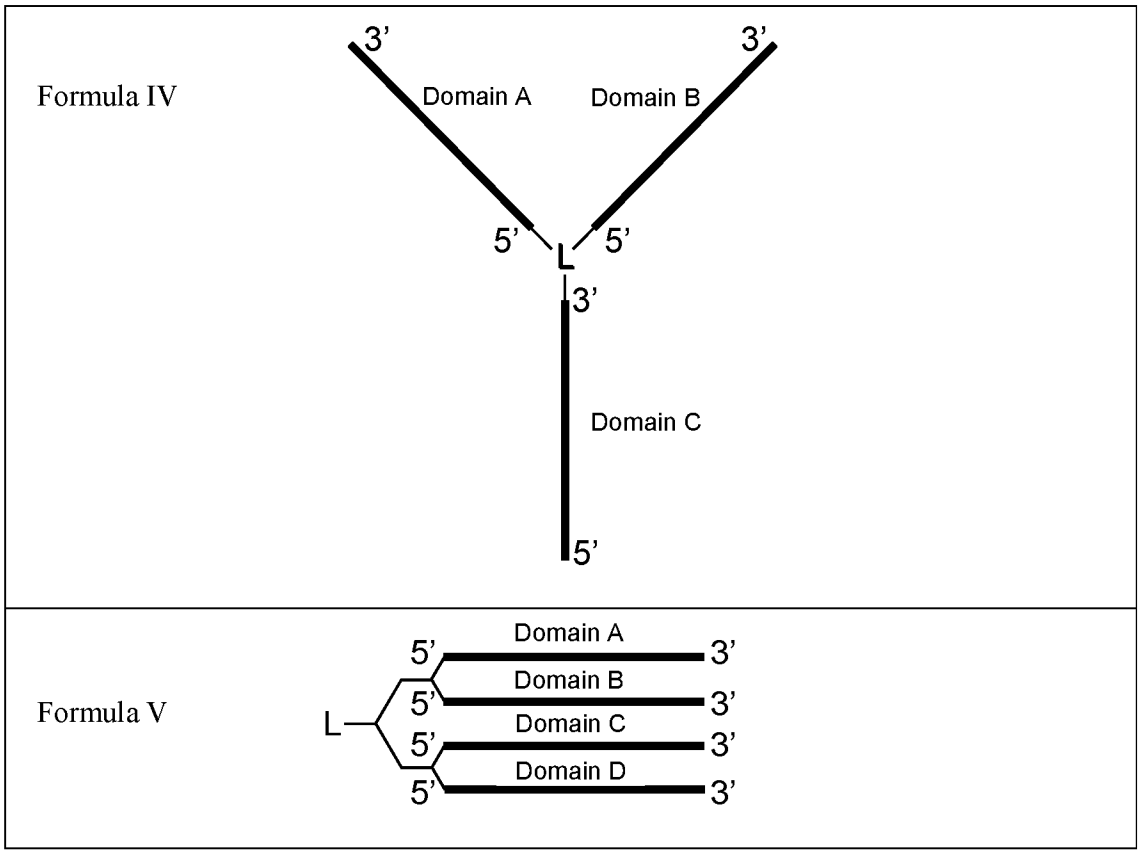
[00116] In some embodiments, the small molecule linker is glycerol or a glycerol homolog of the formula HO-$(CH_2)_o$-CH(OH)-$(CH_2)_p$-OH, wherein *o* and *p* independently are integers from 1 to about 6, from 1 to about 4 or from 1 to about 3. In some other embodiments, the small molecule linker is a derivative of 1,3-diamino-2-hydroxypropane.

Some such derivatives have the formula $HO\text{-}(CH_2)_m\text{-}C(O)NH\text{-}CH_2\text{-}CH(OH)\text{-}CH_2\text{-}NHC(O)\text{-}(CH_2)_m\text{-}OH$, wherein *m* is an integer from 0 to about 10, from 0 to about 6, from 2 to about 6 or from 2 to about 4.

[00117] In certain embodiments, the two or more oligonucleotides of the gene silencing compounds of the invention can be linked as shown in Table 2.

Table 2: Oligoribonucleotide Formulas II – V

Formula II	
Formula III	



[00118] In certain embodiments of Formulas II and/or V, L is a linker or a nucleotide linkage and Domain A and/or Domain B are antisense oligonucleotides that are designed to selectively hybridize to the same target RNA sequence or different target RNA sequences.

[00119] In certain embodiments of Formulas II, III, IV or V, L is a linker and Domain A and/or Domain B and/or Domain C and/or Domain D are antisense oligonucleotides that are designed to selectively hybridize to the same target RNA sequence or different target RNA sequences. For example, in one embodiment, Domain A and/or Domain B and/or Domain C of Formulas II and/or III are antisense oligonucleotides that are designed to selectively hybridize to the same target RNA sequence. In this embodiment, Domain A and/or Domain B and/or Domain C can be designed to hybridize to the same region on the target RNA sequence or to different regions of the same target RNA sequence.

[00120] In a further embodiment of this aspect of the invention, Domain A, Domain B, Domain C, and Domain D are independently RNA or DNA-based oligonucleotides. In certain aspects of this embodiment, the oligonucleotides comprise mixed backbone oligonucleotides.

[00121] In another embodiment, one or more of Domain A and/or Domain B and/or Domain C and/or Domain D is an antisense oligonucleotide that is designed to selectively hybridize to one target RNA sequence and one or more of the remaining Domain A and/or Domain B and/or Domain C and/or Domain D is an antisense oligonucleotide that is designed to selectively hybridized to a different target RNA sequence.

[00122] In another embodiment, one or more of Domain A and/or Domain B and/or Domain C and/or Domain D is an RNA-based oligonucleotide hybridized to a complimentary RNA-based oligonucleotide such that the domain comprises an siRNA molecule.

[00123] These gene silencing compounds of the invention can be prepared by the art recognized methods such as phosphoramidate or H-phosphonate chemistry which can be carried out manually or by an automated synthesizer. The synthetic antisense oligonucleotides of the invention may also be modified in a number of ways without compromising their ability to hybridize to mRNA. Such modifications may include at least one internucleotide linkage of the oligonucleotide being an alkylphosphonate, phosphorothioate, phosphorodithioate, methylphosphonate, phosphate ester, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate hydroxyl, acetamidate or carboxymethyl ester or a combination of these and other internucleotide linkages between the 5' end of one nucleotide and the 3' end of another nucleotide in which the 5' nucleotide phosphodiester linkage has been replaced with any number of chemical groups.

[00124] The synthetic antisense oligonucleotides of the invention may comprise combinations of internucleotide linkages. For example, U.S. Pat. No. 5,149,797 describes traditional chimeric oligonucleotides having a phosphorothioate core region interposed between methylphosphonate or phosphoramidate flanking regions. Additionally, U.S. Pat. No. 5,652,356 discloses "inverted" chimeric oligonucleotides comprising one or more nonionic oligonucleotide region (e.g. alkylphosphonate and/or phosphoramidate and/or phosphotriester internucleoside linkage) flanked by one or more region of oligonucleotide phosphorothioate. Various synthetic antisense oligonucleotides with modified internucleotide linkages can be prepared according to standard methods. In certain embodiments, the phosphorothioate linkages may be mixed Rp and Sp enantiomers, or they may be made stereoregular or substantially stereoregular in either Rp or Sp form.

[00125] Other modifications of gene silencing compounds of the invention include those that are internal or at the end(s) of the oligonucleotide molecule and include additions

to the molecule of the internucleoside phosphate linkages, such as cholesterol, cholesteryl, or diamine compounds with varying numbers of carbon residues between the amino groups and terminal ribose, deoxyribose and phosphate modifications which cleave, or crosslink to the opposite chains or to associated enzymes or other proteins which bind to the genome. Examples of such modified oligonucleotides include oligonucleotides with a modified base and/or sugar such as 2'-O,4'-C-methylene-b-D-ribofuranosyl, or arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide having a sugar which, at both its 3' and 5' positions, is attached to a chemical group other than a hydroxyl group (at its 3' position) and other than a phosphate group (at its 5' position).

[00126] Other examples of modifications to sugars of the oligonucleotide-based compounds of the invention include modifications to the 2' position of the ribose moiety which include but are not limited to 2'-O-substituted with an –O-alkyl group containing 1-6 saturated or unsaturated carbon atoms, or with an –O-aryl, or –O-allyl group having 2-6 carbon atoms wherein such –O-alkyl, -O-aryl or –O-allyl group may be unsubstituted or may be substituted, for example with halo, hydroxyl, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxy, carbalkoxyl or amino groups. None of these substitutions are intended to exclude the presence of other residues having native 2'-hydroxyl group in the case of ribose or 2' H- in the case of deoxyribose.

[00127] The gene silencing compounds according to the invention can comprise one or more ribonucleotides. For example, US Pat No. 5,652,355 discloses traditional hybrid oligonucleotides having regions of 2'-O-substituted ribonucleotides flanking a DNA core region. U.S. Pat. No. 5,652,356 discloses an "inverted" hybrid oligonucleotide that includes an oligonucleotide comprising a 2'-O-substituted (or 2' OH, unsubstituted) RNA region which is in between two oligodeoxyribonucleotide regions, a structure that "inverted relative to the "traditional" hybrid oligonucleotides. Non-limiting examples of particularly useful oligonucleotides of the invention have 2'-O-alkylated ribonucleotides at their 3', 5', or 3' and 5' termini, with at least four, and in some exemplary embodiments five, contiguous nucleotides being so modified. Non-limiting examples of 2'-O-alkylated groups include 2'-O-methyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-butyls and 2'-O-methoxy-ethyl.

[00128] The oligonucleotide-based compounds of the invention may conveniently be synthesized using an automated synthesizer and phosphoramidite approach further described in Example 1. In some embodiments, the oligonucleotide-based compounds of the invention are synthesized by a linear synthesis approach.

[00129] An alternative mode of synthesis is "parallel synthesis", in which synthesis proceeds outward from a central linker moiety. A solid support attached linker can be used for parallel synthesis, as is described in U.S. Patent No. 5,912,332. Alternatively, a universal solid support (such as phosphate attached controlled pore glass) support can be used.

[00130] Parallel synthesis of the oligonucleotide-based compounds of the invention has several advantages over linear synthesis: (1) parallel synthesis permits the incorporation of identical monomeric units; (2) unlike in linear synthesis, both (or all) the monomeric units are synthesized at the same time, thereby the number of synthetic steps and the time required for the synthesis is the same as that of a monomeric unit; and (3) the reduction in synthetic steps improves purity and yield of the final immune modulatory oligoribonucleotide product.

[00131] At the end of the synthesis by either linear synthesis or parallel synthesis protocols, the oligonucleotide-based compounds of the invention may conveniently be deprotected with concentrated ammonia solution or as recommended by the phosphoramidite supplier, if a modified nucleoside is incorporated. The product oligonucleotide-based compounds is preferably purified by reversed phase HPLC, detritylated, desalted and dialyzed.

[00132] In certain embodiments, the oligonucleotides of the gene silencing compound according to the invention are selected from the non-limiting list of the oligonucleotides shown in Table 3 below. The oligonucleotides shown in Table 3 have phosphorothioate (PS) linkages, but may also include phosphodiester linkages. Those skilled in the art will recognize, however, that other linkages, based on phosphodiester or non-phosphodiester moieties may be included.

Table 3

Oligo # / SEQ ID NO:	Target	Species	Target Site	Sequence 5' → 3'
1	PD1	Mouse	58	GCCGGACCCACATGCCCAG
2	PD1	Mouse	65	GGTACCTGCCGGACCCACA
3	PD1	Mouse	115	GCCACCCTGATTGCCAGCT
4	PD1	Mouse	198	GGTGGCATTTGCTCCCTCT
5	PD1	Mouse	755	GGTGTCTTCTCTCGTCCCT
6	PD1	Mouse	848	GCTGAGCCCCTACGTCCCA
7	PD1	Mouse	1161	CCCCAGCTCTGCACCTTGT
8	PD1	Mouse	1589	CTAGCTCTGCTGGTTCCCT
9	PD1	Human	69	GCGCCTGTGGGATCTGCAT
10	PD1	Human	108	GCCAGCCCAGTTGTAGCAC
11	PD1	Human	285	GCTTGTCCGTCTGGTTGCT
12	PD1	Human	495	CCCTTCTCTCTGTCACCCT
13	PD1	Human	496	GCCCTTCTCTCTGTCACCC
14	PD1	Human	497	TGCCCTTCTCTCTGTCACC
15	PD1	Human	616	GCCAGGACCCAGACTAGCA
16	PD1	Human	620	GACGGCCAGGACCCAGACT
17	PD1	Human	895	CCATCCTCAGGCCTCAGTG
18	PD1	Human	897	GTCCATCCTCAGGCCTCAG
19	PD1	Human	899	GTGTCCATCCTCAGGCCTC
20	PD1	Human	901	CAGTGTCCATCCTCAGGCC
21	PD1	Human	1003	GCACCCTGCCTGCTTCTCC
22	PD1	Human	1005	CTGCACCCTGCCTGCTTCT
23	PD1	Human	1137	GTGACACCTGCTGCCTGGG
24	PD1	Human	1161	ATCTGGCCCTCCCTGTAGG
25	PD1	Human	1163	GCATCTGGCCCTCCCTGTA
26	PD1	Human	1165	CTGCATCTGGCCCTCCCTG
27	PD1	Human	1167	GACTGCATCTGGCCCTCCC
28	PD1	Human	1169	GTGACTGCATCTGGCCCTC
29	PD1	Human	1412	CTCCTGTGCCCAGTCTTGG
30	PD1	Human	1512	CCCACCACAGCCAGGAGCT
31	PD1	Human	1513	GCCCACCACAGCCAGGAGC
32	PD1	Human	1563	GCCTGAGGTGCTGCCTGGG
33	PD1	Human	1591	CTGCCTCAGCTTCCCTGCC
34	PD1	Human	1592	ACTGCCTCAGCTTCCCTGC
35	PD1	Human	1615	CCTCCAGCTCTGCCTGCCC
36	PD1	Human	1616	GCCTCCAGCTCTGCCTGCC
37	PD1	Human	1720	GCCCTCCTGACCTTGGGAC
38	PD1	Human	1722	CTGCCCTCCTGACCTTGGG
39	PD1	Human	1724	CCCTGCCCTCCTGACCTTG
40	PD1	Human	1894	CCTTCCCACCCAGGCCCTG

41	PD1	Human	1896	TACCTTCCCACCCAGGCCC
42	PD1	Human	1898	TGTACCTTCCCACCCAGGC
43	PD1	Human	1900	CCTGTACCTTCCCACCCAG
44	PD1	Human	1996	CTGGATGCTGGTGGCCCTG
45	PD1	Human	1997	CCTGGATGCTGGTGGCCCT
46	PD1	Human	2024	CCCAGCCACTCAGGTGCCT
47	PD1	Human	2032	TCCCTTGTCCCAGCCACTC
48	PD1	Human	2034	GATCCCTTGTCCCAGCCAC
49	PDL1	Mouse	219	CAAGCAGGTCCAGCTCCCG
50	PDL1	Mouse	316	CTCCCCCTGAAGTTGCTGT
51	PDL1	Mouse	436	TTGTAGTCCGCACCACCGT
52	PDL1	Mouse	1399	GGTGACCTCTGTGTTCCCT
53	PDL1	Mouse	2152	GCCTGCCTCTGCCTCCCTA
54	PDL1	Mouse	3311	GCCCAGCCTGTTCCTTCAG
55	PDL1	Human	571	GGTAGCCCTCAGCCTGACA
56	PDL1	Human	892	CCATCATTCTCCCTTTTCT
57	PDL1	Human	1075	ATTGCCTGCATCCCACGGG
58	PDL1	Human	1080	CCCACATTGCCTGCATCCC
59	PDL1	Human	1103	TTCAGTGCTTGGGCCTTTT
60	PDL1	Human	1163	GGCTCCCTGTTTGACTCCA
61	PDL1	Human	1182	GTATCAAGGTCTCCCTCCA
62	PDL1	Human	1230	TCCTTTCTCCCTGTCACAG
63	PDL1	Human	1296	ATTCTCAACCCGTCTTCCT
64	PDL1	Human	1855	TCTGTTTGCTTCCTCAGCT
65	PDL1	Human	1904	GGGTGGCAGTCTGAGGTCT
66	PDL1	Human	1911	GGACAGTGGGTGGCAGTCT
67	PDL1	Human	2142	TTCCCCTCGCATCATCCTT
68	PDL1	Human	2192	TCCCAGACCACATTGGCCT
69	PDL1	Human	2901	TGCACCCTGGAGAGCCCAT
70	PDL1	Human	3128	GCTGGTGGCATTCAAGGGT
71	PDL1	Human	3173	CGAAACCTCCAGGAAGCCT
72	PDL1	Human	3196	GATCTCCCAGGGCATCTGA
73	PDL1	Human	3397	GCCTTGCTCAGCCACAATT
74	PDL1	Human	3402	TATGTGCCTTGCTCAGCCA
75	IDO1	Mouse	138	CTAGCCACAAGGACCCAGG
76	IDO1	Mouse	264	ATGTACCCCAGGGCCAGGT
77	IDO1	Mouse	295	ATCCCCTCGGTTCCACACA
78	IDO1	Mouse	492	CCCTTGTCGCAGTCCCCAC
79	IDO1	Mouse	817	GAAGATGCTGCTCTGGCCT
80	IDO1	Mouse	1145	CAGTCCCTCTGCTTTCCAC
81	IDO1	Human	172	GCAGAGCAAAGCCCACTTC
82	IDO1	Human	184	CCTGTGGATTTGGCAGAGC
83	IDO1	Human	388	CTCCATGACCTTTGCCCCA
84	IDO1	Human	507	CTTTTTCTTCCAGTTTGCC
85	IDO1	Human	619	CAGCTGCTATTTCCACCAA

86	IDO1	Human	816	GTTGCCTTTCCAGCCAGAC
87	IDO1	Human	823	GCTGGGGGTTGCCTTTCCA
88	IDO1	Human	849	CCCTTCATACACCAGACCG
89	IDO1	Human	956	TGTCCTCCACCAGCAGTCT
90	IDO1	Human	1138	GCAGATGGTAGCTCCTCAG
91	IDO1	Human	1187	TCCTTTGGCTGCTGGCTTG
92	IDO1	Human	1239	GCCTCCAGTTCCTTTGGCT
93	IDO1	Human	1246	AATCAGTGCCTCCAGTTCC
94	IDO1	Human	1327	GTGCTCTTGTTGGGTTACA
95	IDO1	Human	1627	GCCTCGGCCTCCCAAAGTG
96	IDO1	Human	1745	TAGCTGGGACTACAGGTGC
97	IDO1	Human	1767	TCTCCTGCCTCAGCCTCCC
98	IDO1	Human	1774	ACGCCATTCTCCTGCCTCA
99	IDO1	Human	1792	GCTCCGCCTCCCAGGTTCA
100	IDO1	Human	1815	GGCACAATCTTGGCTCACT
101	LAG3	Mouse	25	GCTCCTCCAGACCCAGTCC
102	LAG3	Mouse	321	GGCCTCCCCAGCCCTCCAA
103	LAG3	Mouse	355	GGAGCAGGTCCTCCCTCAT
104	LAG3	Mouse	422	AGCTCTTTCCCAGGCCCTG
105	LAG3	Mouse	585	CCCCTGGTGAAGGTCAAGG
106	LAG3	Mouse	590	GGCATCCCCTGGTGAAGGT
107	LAG3	Mouse	601	GTCTAGGCGAGGGCATCCC
108	LAG3	Mouse	953	GGCACTCGGTTCTGGCCCT
109	LAG3	Mouse	1044	GACACAGCCCCAGGTCCCA
110	LAG3	Mouse	1108	GCTCCAGACCCAGAACCTT
111	LAG3	Mouse	1161	GGGCAGCTCCACCCTAGAA
112	LAG3	Mouse	1260	GCCACTCTTTCCAGCCACG
113	LAG3	Mouse	1295	GCCAGACCCACAGCCTCAA
114	LAG3	Mouse	1316	CAGGTGTAGGTCCCAGCCT
115	LAG3	Mouse	1349	GCATTGAGCTGCTGTCCCT
116	LAG3	Mouse	1524	GGCCTCCTGAATCTCCAGC
117	LAG3	Mouse	1573	GCCTCTGGCCCTCGTACAG
118	LAG3	Mouse	1819	CCAGCTCCTCTATCTTCCT
119	LAG3	Mouse	1918	CTGCCTCGGCTCCAGGTCA
120	LAG3	Mouse	1936	GCTGCTGAGACCTGCTGGC
121	LAG3	Mouse/Human	1315	AGGTGTAGGTCCCAGCCTG
122	LAG3	Mouse/Human	1822	GCTCCAGCTCCTCTATCTT
123	LAG3	Mouse/Human	1062	GCCATCTCTGTAGGTGAGG
124	LAG3	Mouse/Human	1356	GACAGTGGCATTGAGCTGC
125	LAG3	Human	3	TCTCTGGGCCTTCACCCCT
126	LAG3	Human	123	CTGGGCAGATCAGGCAGCC
127	LAG3	Human	167	GGGAGGGATGACCAGAGGC
128	LAG3	Human	229	GGGAGGTGGAGGAAGGGGT
129	LAG3	Human	346	CTGAGCCTCCCACATCTCT
130	LAG3	Human	395	GCTTCACTGGAGCCACCCA

131	LAG3	Human	494	GGCTGAGATCCTGGAGGGG
132	LAG3	Human	524	GCTGCCAAGTGACCCCTGC
133	LAG3	Human	648	GGACCCACGCTCAGCACCG
134	LAG3	Human	736	CCATAGCGAGAAGTCCCCG
135	LAG3	Human	834	TGGCCCAGGCGCAGACGGA
136	LAG3	Human	1034	CCATGGGGCTGACTTGGGG
137	LAG3	Human	1359	TTGAGCTGCTGTTCCTGCA
138	LAG3	Human	1433	GCAGCTTCCCCAGGGATCC
139	LAG3	Human	1499	GGGATGGGGTGTCCAGAGA
140	LAG3	Human	1554	TGGGAAAGGAGCTGGGCCT
141	LAG3	Human	1593	AGAAGCCTCTCCCCCTGGT
142	LAG3	Human	1636	GGCACCTGGGCTAGACAGC
143	LAG3	Human	1848	GGTTCTTGCTCCAGCTCCT
144	LAG3	Human	1940	GCTGAGATCTGCTGGCTGC
145	LAG3	Human/Mouse	1972	GCTGCTGACAGGGAGTTTA
146	LAG3	Human/Mouse	642	ACGCTCAGCACCGTGTAGC
147	LAG3	Human/Mouse	1234	AGGAGGAGTCCACTTGGCA
148	LAG3	Human/Mouse	1366	AGTGGCATTGAGCTGCTGT
149	TIM3	Mouse	222	AATCCCTTGCCCCAGCACA
150	TIM3	Mouse	319	GAGATCGCCCTTTAGCTGG
151	TIM3	Mouse	386	TGCAGCAGTAGGTCCCATG
152	TIM3	Mouse	462	GGAGTGACCTTGGCTGCTT
153	TIM3	Mouse	661	CCCAGCAGAGACTCCCACT
154	TIM3	Mouse	782	CATTTGCCAACCCTCCTGG
155	TIM3	Mouse	887	GCTGGCTGTTGACGTAGCA
156	TIM3	Mouse	1273	TTAGCCCTTTATTCCCCCT
157	TIM3	Mouse	1416	CCTCCTGCCTAAGGTTCCC
158	TIM3	Mouse	1425	ACTTATCACCCTCCTGCCT
159	TIM3	Mouse	1517	GAGCCTCATCTCCAGCCTC
160	TIM3	Mouse	1526	TCACTGTCCGAGCCTCATC
161	TIM3	Mouse	1668	CTGACTGCACGCAAGCCCC
162	TIM3	Mouse	1767	GAGCAGAGGACAACCCCCA
163	TIM3	Mouse	1953	CTGCTCTGCCATGCTCCCA
164	TIM3	Mouse	2138	GTCAGTTCCCCTTGAGCAC
165	TIM3	Mouse	2220	CTGCCTTCGTATGTCCCAG
166	TIM3	Mouse	2461	CACAGTTGCTCCCCAATGC
167	TIM3	Mouse	2570	AGCCAGGACCTCCACAGCT
168	TIM3	Mouse	2596	GTCTCCCTTCCATACCCAC
169	TIM3	Human	59	CTGCCAGGTCTACAGTCAC
170	TIM3	Human	281	CAGCAGCAGCAGCAGGACA
171	TIM3	Human	338	GGCATTCTGACCGACCTCC
172	TIM3	Human	457	TCCCTTTCATCAGTCCTGA
173	TIM3	Human	740	GAGGCTCCCCAGTGTCTGT
174	TIM3	Human	803	GGCCAATCTAGAGTCCCGT
175	TIM3	Human	1110	GTGAGGGTTGCTGCCTGCT

176	TIM3	Human	1235	GCAGTGGACAGAACCTCCA
177	TIM3	Human	1304	CAGTGCAGGTCCCAGTTCA
178	TIM3	Human	1442	GAGCTCCAGAGACCCCACG
179	TIM3	Human	1456	GCCCGAATTTCCTGGAGCT
180	TIM3	Human	1506	CAGCACCCAGTTTTCCCTA
181	TIM3	Human	1549	GCCCCTTTAGACTTTCTGT
182	TIM3	Human	1640	TGCCATTGCACTCCAGCCT
183	TIM3	Human	1716	ATCCCAGCCACTCAGGAGG
184	TIM3	Human	1725	ATGCCTGTAATCCCAGCCA
185	TIM3	Human	1863	GCTCACGCCTGTAATCCCA
186	TIM3	Human	1877	GGCTGGATGTGGTGGCTCA
187	TIM3	Human	2053	GCCACATCTCAGCCCTGCA
188	TIM3	Human	2246	GCCTTTGCCTTCTTTCCAC
189	CTLA4	Mouse	106	GGTCCTCAGGGAGCAGAGT
190	CTLA4	Mouse	191	AGGCCAAGTCCTAGAAGGC
191	CTLA4	Mouse	253	TGGGTCACCTGTATGGCTT
192	CTLA4	Mouse	344	AGTCACCCGGACCTCATCA
193	CTLA4	Mouse	416	GCCCACTGTATTCTTCTCT
194	CTLA4	Mouse	497	GTCAACAGCTCTCAGTCCT
195	CTLA4	Mouse	563	GTTGCCCATGCCCACAAAG
196	CTLA4	Mouse	567	TCCCGTTGCCCATGCCCAC
197	CTLA4	Mouse	647	CCCCAAGCTAACTGCGACA
198	CTLA4	Mouse	735	TCACATAGACCCCTGTTGT
199	CTLA4	Mouse	760	CATTCTGGCTCTGTTGGGG
200	CTLA4	Mouse	1084	CCTTGACCCCACACCATAA
201	CTLA4	Mouse	1135	CTCTTCCTTCACCCCCTTC
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203	CTLA4	Mouse	1436	AGCTCCCCAGCCAAACCTC
204	CTLA4	Mouse	1470	GACCTCGAGTCCAACCTGA
205	CTLA4	Mouse	1484	GCCAGTTGGTGCAGGACCT
206	CTLA4	Mouse	1542	ACTCCATCACCATCGGTTT
207	CTLA4	Mouse	1552	CCCAGTTTACACTCCATCA
208	CTLA4	Mouse	1794	TCCCATCCTACCATCTGCT
209	CTLA4	Human	129	GGGAGCGGTGTTCAGGTCT
210	CTLA4	Human	211	AGGAGAGTGCAGGGCCAGG
211	CTLA4	Human	346	CGGACCTCAGTGGCTTTGC
212	CTLA4	Human	504	CCATGGCCCTCAGTCCTTG
213	CTLA4	Human	574	CCGTTGCCTATGCCCAGGT
214	CTLA4	Human	953	GGGTTCCGCATCCAACTTT
215	CTLA4	Human	1007	CATCCCAGCTCTGTCTTTC
216	CTLA4	Human	1067	GCATCCCCATATTAATCCC
217	CTLA4	Human	1136	CTCCCTGCCTTTTCCTTCT
218	CTLA4	Human	1308	ACCTTTAGCATCACTGGCT
219	CTLA4	Human	1514	AGTGTCCTGAGCTCCTCCA
220	CTLA4	Human	1537	CCTTGTGTTCTACCTGGTG

221	CTLA4	Human	1570	CCTCATCCAGTTTCCAAGC
222	CTLA4	Human	1606	CTCAGCACAATTCCACGCA
223	CTLA4	Human	1632	AGCCCCAAAGCACATGTCA
224	CTLA4	Human	1747	ATACCTGTGGGTCTCCTGG
225	CTLA4	Human	1822	GCCTTCTTCTGTCCATGGC
226	CTLA4	Human	1844	GCACCCCATTCTGCCACCT
227	CTLA4	Human/Mouse	744	TCACATAGACCCCTGTTGT
228	CTLA4	Human/Mouse	1117	TTGGGCTGTGCCATTCCCT
229	IDO2	Mouse	49	TGCCCCAGAGGAATGCCCA
230	IDO2	Mouse	127	GTGGTATCTCCCCAAGGAC
231	IDO2	Mouse	279	CAGTCCAGGAGAGGCATCC
232	IDO2	Mouse	440	GGAGTCCCAAGTTCCTGGA
233	IDO2	Mouse	510	TCCAACGGTCCTTCTGGGT
234	IDO2	Mouse	639	GCCTCCATTCCCTGAACCA
235	IDO2	Mouse	801	GGATTGTCCTTCCACCCAG
236	IDO2	Mouse	873	GCTGCACTTCCTCCAGAGT
237	IDO2	Mouse	971	GCGGCATGTAGTCCCTCAT
238	IDO2	Mouse	1047	CCAGGACCAGAGGCCAGTA
239	IDO2	Mouse	1215	GTACCCCCAGTGCCCCTGT
240	IDO2	Mouse	1280	CACCAGGACACAGGAGGGC
241	IDO2	Mouse	1617	GCTCCCACGGGACCTGACT
242	IDO2	Mouse	1782	TGAGGAGGTCATGGCTGCA
243	IDO2	Mouse	1911	GGGACGAGGGAGGTAGGGA
244	IDO2	Mouse	2058	GTTTGAGGCCCATCAGACC
245	IDO2	Mouse	2345	GCTCAGTGGCTCATCCCTG
246	IDO2	Mouse	2638	GGCTGTCCCAGGTCACAGA
247	IDO2	Mouse	2748	GGTGACTTCCAGGTCTGCA
248	IDO2	Mouse	2756	CCCGTGCTGGTGACTTCCA
249	IDO2	Human	156	GGTGTCCATTGCCTTCTGT
250	IDO2	Human	214	GCCTGGTGGGTGAAGTGTC
251	IDO2	Human	222	TTGTGGTGGCCTGGTGGGT
252	IDO2	Human	284	ATTCGGTCTGTGGGGCTCC
253	IDO2	Human	561	CTCCTTCCTGCCAGACATA
254	IDO2	Human	633	GCCCCAAGTTCCTGGAGAC
255	IDO2	Human	713	CCCAATTTCCAGGAATCCG
256	IDO2	Human	722	CTCCAGGTTCCCAATTTCC
257	IDO2	Human	757	TGCAGGCTCTCTCCCCCAG
258	IDO2	Human	802	GGCACTGCTTCTTTCTCTA
259	IDO2	Human	1137	AGTCACCACTTTCCTTGCT
260	IDO2	Human	1207	GGTGCTGAGTGGATGTCTT
261	IDO2	Human	1253	CAGCAAGTGGTCCTGTCCA
262	IDO2	Human	1363	GGCTTCCCATGCTTTGCCT
263	IDO2	Human	1415	TCCACCTGTGCCCCTGTCT
264	IDO2	Human	1464	ACTCCAAGGTCTTATCCCT
265	IDO2	Human	1573	TGATCCCAGGCAGAACCCT

266	IDO2	Human	1593	GGGCTGAGATCCTTCCTGG
267	IDO2	Human	1745	TGGGGGTTCTGCATGAGGA
268	IDO2	Human	1752	ACTCCTCTGGGGGTTCTGC
269	IDO2	Human	1837	AGTAATGTATCCCCAGGCA
270	IDO2	Human	1945	AAGAGGGCTGGTCTGGGAC
271	CEACAM1	Mouse	291	GTAGTGTTTCCCTTGTACC
272	CEACAM1	Mouse	294	GCCGTAGTGTTTCCCTTGT
273	CEACAM1	Mouse	299	CTATAGCCGTAGTGTTTCC
274	CEACAM1	Mouse	1110	GTGAGGAACAGAATCCGGG
275	CEACAM1	Mouse	1526	TTCCTGCTTCTGGTTTGTT
276	CEACAM1	Mouse	1530	CCATTTCCTGCTTCTGGTT
277	CEACAM1	Mouse	1531	GCCATTTCCTGCTTCTGGT
278	CEACAM1	Mouse	2474	CCATGCTGGAACTCTGTCT
279	CEACAM1	Mouse	2485	CTGCACAGGCTCCATGCTG
280	CEACAM1	Mouse	2486	CCTGCACAGGCTCCATGCT
281	CEACAM1	Mouse	2500	CTGTGGGATTGAAACCTGC
282	CEACAM1	Mouse	2507	GGTGTTACTGTGGGATTGA
283	CEACAM1	Mouse	2513	GCAGAAGGTGTTACTGTGG
284	CEACAM1	Mouse	2533	GTCTGAGCAGGTGGGGTGC
285	CEACAM1	Mouse	2536	GCAGTCTGAGCAGGTGGGG
286	CEACAM1	Mouse	2568	TGTCCAGGTAGCCAGGCCT
287	CEACAM1	Mouse	2570	AATGTCCAGGTAGCCAGGC
288	CEACAM1	Human	103	GCCCTGTCTTCACCTGTGG
289	CEACAM1	Human	111	TCCTGCTGGCCCTGTCTTC
290	CEACAM1	Human	126	GTGCCCCATGGTGTCTCCT
291	CEACAM1	Human	1021	TGGCGTGGCAGGTATAGGA
292	CEACAM1	Human	1403	GCCCCAGGTGAGAGGCCAT
293	CEACAM1	Human	1440	AACCAGGGCCACTACTCCA
294	CEACAM1	Human	1463	GCCAGGGCTACTGCTATCA
295	CEACAM1	Human	1851	GGTTTCCTACAGACTCCCA
296	CEACAM1	Human	1908	GTTCTGGTCCCTCTTTCCC
297	CEACAM1	Human	2230	GGTGCTTAGACCCTGATCC
298	CEACAM1	Human	2396	CTGCCTTGAACAGAGCCCA
299	CEACAM1	Human	2414	AACCCCTCCCTCTCAGCAC
300	CEACAM1	Human	2436	GCTGGTTCCCTCCTGAAGC
301	CEACAM1	Human	2473	CCTTTCCCAAGTTCCTAGC
302	CEACAM1	Human	2489	GGGCAGCTCTCTGATTCCT
303	CEACAM1	Human	2700	GCTCCTGACCAAGGGACCT
304	CEACAM1	Human	2894	AGCAGAGGCCAAGGTTTCC
305	CEACAM1	Human	2924	CTCCCACTTCTCAAGGACC
306	CEACAM1	Human	3019	TCACAGCCCCATTTCCCCA
307	CEACAM1	Human	3323	GCACAGTCCGTGTCAGGGT
308	OX40	Mouse	20	GTATGCAGAGTCCCATGAT
309	OX40	Mouse	121	CCTTGCAGGGTGTGGCTAT
310	OX40	Mouse	161	CCTTGTCTGCTTTCTGCCT

311	OX40	Mouse	270	TGTGACCACTGGGGTAGGT
312	OX40	Mouse	495	GAGGTTGGGTGCCTGGTCT
313	OX40	Mouse	509	GCCGCTGTCCTGCCGAGGT
314	OX40	Mouse	544	GGAGGGCAGGGAACACAGT
315	OX40	Mouse	572	CTGGTTGTTGCCTGGAGAA
316	OX40	Mouse	593	ATTGGTCCAGGGCTTGCAG
317	OX40	Mouse	642	CCAAGCTGTCACTGGCTGG
318	OX40	Mouse	693	GGGTCTCCCAGAGCAGTGT
319	OX40	Mouse	845	AGTCAAGGGAGCCAGCAGG
320	OX40	Mouse	904	GGTTTGGGAGTGTTAGGCA
321	OX40	Mouse	941	CTCCTGGATCGGGGTCCTG
322	OX40	Mouse	1010	GCCCCATAAAATCCACTCC
323	OX40	Mouse	1021	GGGTTGTCCGTGCCCCATA
324	OX40	Mouse	1038	GGCAGGCATCAGGATATGG
325	OX40	Mouse	1069	GCCCAGCACCTAGAACGGT
326	OX40	Mouse	1080	GCCCAGAGCCAGCCCAGCA
327	OX40	Mouse	1126	TTAGGAGCACCACCAGGCA
328	OX40	Human	82	CCCAGGAGGAGCAGAGCCG
329	OX40	Human	192	TGCAGCGGCTCACCATCCC
330	OX40	Human	273	AGGGCTTGCACGGCTTGGA
331	OX40	Human	300	TCCCACTTCTGAGGTTACA
332	OX40	Human	312	GCTTCCGCTCACTCCCACT
333	OX40	Human	342	AGACTGTGTCCTGTGTGGC
334	OX40	Human	347	GCGGCAGACTGTGTCCTGT
335	OX40	Human	401	GGCACAGTCAACTCCAGGC
336	OX40	Human	462	AGTTGGTCCAGGGCTTGCA
337	OX40	Human	485	GGTGTGCTTCCCAGCCAAG
338	OX40	Human	746	CCGGAGCAGGTACAGGGCC
339	OX40	Human	762	GCAGCCTCTGGTCCCTCCG
340	OX40	Human	763	GGCAGCCTCTGGTCCCTCC
341	OX40	Human	823	TGCTCCTCTTGGATGGGGG
342	OX40	Human	865	GGCCCAGGTCAGATCTTGG
343	OX40	Human	967	GTTGGCCCAGGAGCGTGGC
344	OX40	Human	1036	GCAGGAGGTATGCATGGCA
345	OX40	Human	1058	GTTTTTATTGTGGTCCCGC
346	OX40	Human	1075	GACTCCCGTCTGCCAAGGT
347	OX40L	Mouse	141	CCCTTCCCCTTCCATCTCT
348	OX40L	Mouse	167	TCCAGATTCTCATCCAGGG
349	OX40L	Mouse	182	GGCCTTGATCCGTTTTCCA
350	OX40L	Mouse	218	ACCACCAGCCTTAGCGTCT
351	OX40L	Mouse	226	TCCCAGAGACCACCAGCCT
352	OX40L	Mouse	240	CCCTGCTCCCTTGATCCCA
353	OX40L	Mouse	303	TGGAGGGTCCTTTGCCGGA
354	OX40L	Mouse	399	GTTCTGCACCTCCATAGTT
355	OX40L	Mouse	454	AGGAGCCCTTCAGGTAGAT

356	OX40L	Mouse	565	CCAAAGAGGCCACCACAGT
357	OX40L	Mouse	650	ACAATCAGCTCCCCATCAT
358	OX40L	Mouse	753	CCTGTGTCCCGTCCACCCT
359	OX40L	Mouse	817	AGGGTAGGCTCTGCATTCA
360	OX40L	Mouse	895	GCAGGCTCAAGGCAATCCT
361	OX40L	Mouse	1069	TGGACACCACCCTTTCCAT
362	OX40L	Mouse	1157	CCCCCATGAGATGAGAGAC
363	OX40L	Mouse	1173	AATCTTCTTTCCAAGCCCC
364	OX40L	Mouse	1193	AGTCCTGCTTTCCACGGGG
365	OX40L	Mouse	1298	GGTGGGTATCATAGTCCCT
366	OX40L	Mouse	1439	CCTTCTTGGCCTTTATCCT
367	OX40L	Human	494	GGGCTCCTCATCCTTCTGG
368	OX40L	Human	712	GTTCATGCTGGTGCCTGGT
369	OX40L	Human	814	GGGAGGGCCAGGATCTGCT
370	OX40L	Human	1104	CCTTCACTCCTTGCTCCTC
371	OX40L	Human	1120	GATTCATAACCCCACTCCT
372	OX40L	Human	1139	GTTCATACCACCTTTGGCA
373	OX40L	Human	1276	GGCTCTCTTCAAGTCCTGA
374	OX40L	Human	1378	CACATCCCCAGACAGTTCT
375	OX40L	Human	1383	AGCATCACATCCCCAGACA
376	OX40L	Human	1492	GTCCAGTTCCCTGCTATCC
377	OX40L	Human	1569	TGCTTTGCCTGTCTGTGGC
378	OX40L	Human	1577	GCATGTGTTGCTTTGCCTG
379	OX40L	Human	1828	ATTCCATTGAAGCCCTGGC
380	OX40L	Human	2127	CAGCCCTCCACCTTTCTGG
381	OX40L	Human	2367	GTCCACAGTAGGCCCTCCA
382	OX40L	Human	2376	CAGTGCCTGGTCCACAGTA
383	OX40L	Human	2387	AGTATTTAGCCCAGTGCCT
384	OX40L	Human	2729	CCCAAAGCGAGTGAGCACC
385	OX40L	Human	2754	ACATGGGAAGAGCAGGCCA
386	OX40L	Human	2808	GGTGGAGTGAGGCTGGTGC

[00133] Compound names for the 3rd generation antisense (3GA) compounds according to the invention are based on the target and oligonucleotide target site(s) as depicted Table 3. For example, "3GA 384" comprises two copies of Oligo # 384 linked at their 5' ends (e.g., 3'-CCACGAGTGAGCGAAACCC-5'-X-5'-CCCAAAGCGAGTGAGCACC-3', wherein X represents a non-nucleotidic linker). Alternatively, a 3GA compound comprising two different oligonucleotides such as Oligo # 385 and Oligo # 386 (e.g., 3'-ACCGGACGAGAAGGGTACA-5'-X-5'-GGTGGAGTGAGGCTGGTGC-3', wherein X represents a non-nucleotidic linker) will be referred to herein, for example, as "3GA 385/386".

[00134] Certain embodiments provide gene silencing compounds comprising two oligonucleotides independently selected from the oligonucleotides listed in Table 3. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of 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, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 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, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, or combinations thereof. In certain embodiments, the oligonucleotides of the gene silencing compound are the same. In certain embodiments, the oligonucleotides of the gene silencing compounds are different.

[00135] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 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, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 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, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386,and is at least 80% complimentary to its target site with SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389, SEQ ID NO: 390, SEQ ID NO: 391, SEQ ID NO: 392, SEQ ID NO: 393, SEQ ID NO: 394, SEQ ID NO: 395, SEQ ID NO: 396, SEQ ID NO: 397, SEQ ID NO: 398, SEQ ID NO: 399, SEQ ID NO: 400, SEQ ID NO: 401, SEQ ID NO: 402, SEQ ID NO: 403, SEQ ID NO: 404, SEQ ID NO: 405, or SEQ ID NO: 406. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 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, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 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, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246,

247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, and is at least 85% complimentary to its target site with SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389, SEQ ID NO: 390, SEQ ID NO: 391, SEQ ID NO: 392, SEQ ID NO: 393, SEQ ID NO: 394, SEQ ID NO: 395, SEQ ID NO: 396, SEQ ID NO: 397, SEQ ID NO: 398, SEQ ID NO: 399, SEQ ID NO: 400, SEQ ID NO: 401, SEQ ID NO: 402, SEQ ID NO: 403, SEQ ID NO: 404, SEQ ID NO: 405, or SEQ ID NO: 406. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 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, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 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, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380,

381, 382, 383, 384, 385, or 386, and is at least 90% complimentary to its target site with SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389, SEQ ID NO: 390, SEQ ID NO: 391, SEQ ID NO: 392, SEQ ID NO: 393, SEQ ID NO: 394, SEQ ID NO: 395, SEQ ID NO: 396, SEQ ID NO: 397, SEQ ID NO: 398, SEQ ID NO: 399, SEQ ID NO: 400, SEQ ID NO: 401, SEQ ID NO: 402, SEQ ID NO: 403, SEQ ID NO: 404, SEQ ID NO: 405, or SEQ ID NO: 406. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 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, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 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, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386,and is at least 95% complimentary to its target site with SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389, SEQ ID NO: 390, SEQ ID NO: 391, SEQ ID NO: 392, SEQ ID NO: 393, SEQ ID NO: 394, SEQ ID NO: 395, SEQ ID NO: 396, SEQ ID NO: 397, SEQ ID NO: 398, SEQ ID NO: 399, SEQ ID NO: 400, SEQ ID NO: 401, SEQ ID NO: 402, SEQ ID NO: 403, SEQ ID NO: 404, SEQ ID NO: 405, or SEQ ID NO: 406.

[00136] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous

nucleobases of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 49, 50, 51, 52, 53, 54, 75, 76, 77, 78, 79, 80, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, or 365, and is at least 80% complimentary to its target site with SEQ ID NO: 387, SEQ ID NO: 389, SEQ ID NO: 391, SEQ ID NO: 393, SEQ ID NO: 395, SEQ ID NO: 397, SEQ ID NO: 399, SEQ ID NO: 401, SEQ ID NO: 403, or SEQ ID NO: 405. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 49, 50, 51, 52, 53, 54, 75, 76, 77, 78, 79, 80,101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, or 365, and is at least 85% complimentary to its target site with SEQ ID NO: 387, SEQ ID NO: 389, SEQ ID NO: 391, SEQ ID NO: 393, SEQ ID NO: 395, SEQ ID NO: 397, SEQ ID NO: 399, SEQ ID NO: 401, SEQ ID NO: 403, or SEQ ID NO: 405. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 49, 50, 51, 52, 53, 54, 75, 76, 77, 78, 79, 80,101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326,

327, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, or 365, and is at least 90% complimentary to its target site with SEQ ID NO: 387, SEQ ID NO: 389, SEQ ID NO: 391, SEQ ID NO: 393, SEQ ID NO: 395, SEQ ID NO: 397, SEQ ID NO: 399, SEQ ID NO: 401, SEQ ID NO: 403, or SEQ ID NO: 405. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 49, 50, 51, 52, 53, 54, 75, 76, 77, 78, 79, 80,101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, or 365, and is at least 95% complimentary to its target site with SEQ ID NO: 387, SEQ ID NO: 389, SEQ ID NO: 391, SEQ ID NO: 393, SEQ ID NO: 395, SEQ ID NO: 397, SEQ ID NO: 399, SEQ ID NO: 401, SEQ ID NO: 403, or SEQ ID NO: 405.

[00137] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, and is at least 80% complimentary to its target site within SEQ ID NO: 388, SEQ ID NO: 390, SEQ ID NO: 392, SEQ ID NO: 394, SEQ ID NO: 396, SEQ ID NO: 398, SEQ ID NO: 400, SEQ ID NO: 402,

SEQ ID NO: 404, or SEQ ID NO: 406. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, and is at least 85% complimentary to its target site within SEQ ID NO: 388, SEQ ID NO: 390, SEQ ID NO: 392, SEQ ID NO: 394, SEQ ID NO: 396, SEQ ID NO: 398, SEQ ID NO: 400, SEQ ID NO: 402, SEQ ID NO: 404, or SEQ ID NO: 406. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, and is at least 90% complimentary to its target site within SEQ ID NO: 388, SEQ ID NO: 390, SEQ ID NO: 392, SEQ ID NO: 394, SEQ ID NO: 396, SEQ ID NO: 398, SEQ ID NO: 400, SEQ ID NO: 402, SEQ ID NO: 404, or SEQ ID NO: 406. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at

least 12 contiguous nucleobases of SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, and is at least 95% complimentary to its target site within SEQ ID NO: 388, SEQ ID NO: 390, SEQ ID NO: 392, SEQ ID NO: 394, SEQ ID NO: 396, SEQ ID NO: 398, SEQ ID NO: 400, SEQ ID NO: 402, SEQ ID NO: 404, or SEQ ID NO: 406.

[00138] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, or 8, and is at least 80% complimentary to SEQ ID NO: 387. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, or 8, and is at least 85% complimentary to SEQ ID NO: 387. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, or 8, and is at least 90% complimentary to SEQ ID NO: 387. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, or 8, and is at least 95% complimentary to SEQ ID NO: 387.

[00139] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, and is at least 80% complimentary to SEQ ID NO: 388. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at

least 12 contiguous nucleobases of SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, and is at least 85% complimentary to SEQ ID NO: 388. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, and is at least 90% complimentary to SEQ ID NO: 388. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, and is at least 95% complimentary to SEQ ID NO: 388.

[00140] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 49, 50, 51, 52, 53, 54, and is at least 80% complimentary to SEQ ID NO: 389. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 49, 50, 51, 52, 53, 54, and is at least 85% complimentary to SEQ ID NO: 389. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 49, 50, 51, 52, 53, 54, and is at least 90% complimentary to SEQ ID NO: 389. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 49, 50, 51, 52, 53, 54, and is at least 95% complimentary to SEQ ID NO: 389.

[00141] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, and is at least 80% complimentary to SEQ ID NO: 390. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, and is at least 85% complimentary to SEQ ID NO: 390. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12

contiguous nucleobases of SEQ ID NOs: 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, and is at least 90% complimentary to SEQ ID NO: 390. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, and is at least 95% complimentary to SEQ ID NO: 390.

[00142] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 75, 76, 77, 78, 79, 80, and is at least 80% complimentary to SEQ ID NO: 391. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 75, 76, 77, 78, 79, 80, and is at least 85% complimentary to SEQ ID NO: 391. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 75, 76, 77, 78, 79, 80, and is at least 90% complimentary to SEQ ID NO: 391. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 75, 76, 77, 78, 79, 80, and is at least 95% complimentary to SEQ ID NO: 391.

[00143] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, and is at least 80% complimentary to SEQ ID NO: 392. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, and is at least 85% complimentary to SEQ ID NO: 392. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, and is at least 90% complimentary to SEQ ID NO: 392. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, and is at least 95% complimentary to SEQ ID NO: 392.

[00144] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, and is at least 80% complimentary to SEQ ID NO: 393. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, and is at least 85% complimentary to SEQ ID NO: 393. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, and is at least 90% complimentary to SEQ ID NO: 393. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, and is at least 95% complimentary to SEQ ID NO: 393.

[00145] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, and is at least 80% complimentary to SEQ ID NO: 394. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, and is at least 85% complimentary to SEQ ID NO: 394. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, and is at least 90% complimentary to SEQ ID NO: 394. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, and is at least 95% complimentary to SEQ ID NO: 394.

[00146] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, and is at least 80% complimentary to SEQ ID NO: 395. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, and is at least 85% complimentary to SEQ ID NO: 395. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, and is at least 90% complimentary to SEQ ID NO: 395. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, and is at least 95% complimentary to SEQ ID NO: 395.

[00147] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, and is at least 80% complimentary to SEQ ID NO: 396. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, and is at least 85% complimentary to SEQ ID NO: 396. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, and is at least 90% complimentary to SEQ ID NO: 396. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, and is at least 95% complimentary to SEQ ID NO: 396.

[00148] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous

nucleobases of SEQ ID NOs: 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, and is at least 80% complimentary to SEQ ID NO: 397. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, and is at least 85% complimentary to SEQ ID NO: 397. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, and is at least 90% complimentary to SEQ ID NO: 397. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, and is at least 95% complimentary to SEQ ID NO: 397.

[00149] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, and is at least 80% complimentary to SEQ ID NO: 398. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, and is at least 85% complimentary to SEQ ID NO: 398. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, and is at least 90% complimentary to SEQ ID NO: 398. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, and is at least 95% complimentary to SEQ ID NO: 398.

[00150] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, and is at least 80% complimentary to SEQ ID NO:

399. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, and is at least 85% complimentary to SEQ ID NO: 399. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, and is at least 90% complimentary to SEQ ID NO: 399. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, and is at least 95% complimentary to SEQ ID NO: 399.

[00151] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, and is at least 80% complimentary to SEQ ID NO: 400. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, and is at least 85% complimentary to SEQ ID NO: 400. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, and is at least 90% complimentary to SEQ ID NO: 400. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, and is at least 95% complimentary to SEQ ID NO: 400.

[00152] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, and is at least 80% complimentary to SEQ ID NO: 401. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each,

independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, and is at least 85% complimentary to SEQ ID NO: 401. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, and is at least 90% complimentary to SEQ ID NO: 401. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, and is at least 95% complimentary to SEQ ID NO: 401.

[00153] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, and is at least 80% complimentary to SEQ ID NO: 402. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, and is at least 85% complimentary to SEQ ID NO: 402. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, and is at least 90% complimentary to SEQ ID NO: 402. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, and is at least 95% complimentary to SEQ ID NO: 402.

[00154] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, and is at least 80% complimentary to SEQ ID NO: 403. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, and is at least 85% complimentary to SEQ ID NO: 403. In certain

embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, and is at least 90% complimentary to SEQ ID NO: 403. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, and is at least 95% complimentary to SEQ ID NO: 403.

[00155] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, and is at least 80% complimentary to SEQ ID NO: 404. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, and is at least 85% complimentary to SEQ ID NO: 404. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, and is at least 90% complimentary to SEQ ID NO: 404. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, and is at least 95% complimentary to SEQ ID NO: 404.

[00156] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, and is at least 80% complimentary to SEQ ID NO: 405. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, and is at least 85% complimentary to SEQ ID NO: 405. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 347, 348, 349, 350, 351, 352,

353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, and is at least 90% complimentary to SEQ ID NO: 405. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, and is at least 95% complimentary to SEQ ID NO: 405.

[00157] In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, and is at least 80% complimentary to SEQ ID NO: 406. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, and is at least 85% complimentary to SEQ ID NO: 406. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, and is at least 90% complimentary to SEQ ID NO: 406. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, and is at least 95% complimentary to SEQ ID NO: 406.

[00158] In certain embodiments, the invention provides a composition comprising a 3GA compound according to the invention and one or more vaccines, antigens, antibodies, cytotoxic agents, chemotherapeutic agents (both traditional chemotherapy and modern targeted therapies), kinase inhibitors, allergens, antibiotics, agonist, antagonist, antisense oligonucleotides, ribozymes, RNAi molecules, siRNA molecules, miRNA molecules, aptamers, proteins, gene therapy vectors, DNA vaccines, adjuvants, co-stimulatory molecules or combinations thereof.

[00159] In certain embodiments, the invention provides a method for inhibiting PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L mRNA or protein expression, the method comprising contacting a cell with a gene silencing compound

according to the invention. In certain embodiments, the cell can be contacted with two or more gene silencing compounds targeting different regions of the same checkpoint. In certain embodiments, the cell can be contacted with two or more gene silencing compounds targeting different checkpoints.

[00160] Certain embodiments further provide a method to reduce PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or or OX40L mRNA or protein expression in an animal comprising administering to the animal a gene silencing compound or composition as described herein to reduce PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L mRNA or protein expression in the animal. In certain embodiments, the animal is a human. In certain embodiments, reducing PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L mRNA or protein expression prevents, treats, ameliorates, or slows progression of disease. In certain embodiments reducing PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L mRNA or protein expression inhibits immune system tolerance. In certain embodiments two or more gene silencing compounds targeting different regions of the same checkpoint can be administered. In certain embodiments two or more gene silencing compounds targeting different checkpoints can be administered.

[00161] In certain embodiments provided are methods for inhibiting immune system tolerance to tumors comprising administering to the animal a gene silencing compound or composition as described herein to reduce PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L mRNA or protein expression in the animal. In certain embodiments, the animal is a human. In certain embodiments, the gene silencing compound or composition as described herein is administered intratumorally. Thus, the inhibition of PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L mRNA or protein expression may provide a potentially useful immunotherapy strategy for patients with cancer. In certain embodiments two or more gene silencing compounds targeting different regions of the same checkpoint can be administered. In certain embodiments two or more gene silencing compounds targeting different checkpoints can be administered.

[00162] In certain embodiments provided are methods for preventing tumor growth and tumor volume. In certain embodiments provided are methods for reducing tumor growth and tumor volume.

[00163] In certain embodiments provided are methods, compounds, and compositions for the treatment, prevention, or amelioration of diseases, disorders, and conditions associated with PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L in an individual in need thereof. Also contemplated are methods and compounds for the preparation of a medicament for the treatment, prevention, or amelioration of a disease, disorder, or condition associated with PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L. In certain embodiments two or more gene silencing compounds targeting different regions of the same checkpoint can be administered. In certain embodiments two or more gene silencing compounds targeting different checkpoints can be administered.

[00164] PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L associated diseases, disorders, and conditions include hyperproliferative diseases, e.g., cancer, carcinomas, sarcomas, lymphomas, and leukemias as well as associated malignancies and metastases. PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L associated diseases, disorders, and conditions can also include autoimmune diseases and disorders.

[00165] In certain embodiments provided are PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L gene silencing compounds for use in treating, preventing, or ameliorating a PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L associated disease. In certain embodiments, PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L gene silencing compounds are capable of inhibiting the expression of PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L mRNA and/or PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L protein in a cell, tissue, or animal.

[00166] Certain embodiments provide methods comprising administering to an animal a gene silencing compounds as described herein. In certain embodiments two or more gene silencing compounds targeting different regions of the same checkpoint can be administered. In certain embodiments two or more gene silencing compounds targeting different checkpoints can be administered.

[00167] Also provided are methods and gene silencing compounds for the preparation of a medicament for the treatment, prevention, or amelioration of disease.

[00168] Certain embodiments provide the use of gene silencing compounds as described herein in the manufacture of a medicament for treating, ameliorating, or preventing disease.

[00169] Certain embodiments provide gene silencing compounds as described herein for use in treating, preventing, or ameliorating disease as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

[00170] Certain embodiments provide the use of a gene silencing compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating disease as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

[00171] Certain embodiments provide the use of a gene silencing compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating disease as described herein in a patient who is subsequently administered an additional agent or therapy as described herein.

[00172] In any of the methods according to the invention, the gene silencing compound according to the invention can variously act by producing direct gene expression modulation effects alone and/or in combination with any other agent useful for treating or preventing the disease or condition that does not diminish the gene expression modulation effect of the gene silencing compound according to the invention. In any of the methods according to the invention, the agent(s) useful for treating or preventing the disease or condition includes, but is not limited to, vaccines, antigens, antibodies, preferably monoclonal antibodies, cytotoxic agents, kinase inhibitors, allergens, antibiotics, siRNA molecules, antisense oligonucleotides, TLR antagonist (e.g. antagonists of TLR3 and/or TLR7 and/or antagonists of TLR8 and/or antagonists of TLR9), chemotherapeutic agents (both traditional chemotherapy and modern targeted therapies), targeted therapeutic agents, activated cells, peptides, proteins, gene therapy vectors, peptide vaccines, protein vaccines, DNA vaccines, adjuvants, and co-stimulatory molecules (e.g. cytokines, chemokines, protein ligands, trans-activating factors, peptides or peptides comprising modified amino acids), or combinations thereof. For example, in the treatment of cancer, it is contemplated that the oligonucleotide-based compound according to the invention may be administered in combination with one or more chemotherapeutic compound, targeted therapeutic agent and/or

monoclonal antibody. Alternatively, the agent can include DNA vectors encoding for antigen or allergen. Alternatively, the gene silencing compound according to the invention can be administered in combination with other compounds (for example lipids or liposomes) to enhance the specificity or magnitude of the gene expression modulation of the oligonucleotide-based compound according to the invention.

[00173] In any of the methods according to the invention, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, can be by any suitable route, including, without limitation, parenteral, mucosal, oral, sublingual, intratumoral, transdermal, topical, inhalation, intrathecal, intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, by gene gun, dermal patch or in eye drop or mouthwash form. In any of the methods according to the invention, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, can be directly to a tissue or organ such as, but not limited to, the bladder, liver, lung, kidney or lung. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, is by intratumoral administration. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, is by mucosal administration. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, is by oral administration. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, is by intrarectal administration. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, is by intrathecal administration. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, is directly to the bladder. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, is directly to the lung.

[00174] Administration of the therapeutic compositions of gene silencing compounds according to the invention can be carried out using known procedures using an effective amount and for periods of time effective to reduce symptoms or surrogate markers of the disease. For example, an effective amount of a gene silencing compound according to the invention for treating a disease and/or disorder could be that amount necessary to alleviate or reduce the symptoms, or delay or ameliorate the disease and/or disorder. In the context of

administering a composition that modulates gene expression, an effective amount of a gene silencing compound according to the invention is an amount sufficient to achieve the desired modulation as compared to the gene expression in the absence of the gene silencing compound according to the invention. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular compound being administered, the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular compound without necessitating undue experimentation.

[00175] When administered systemically, the therapeutic composition is preferably administered at a sufficient dosage to attain a blood level of gene silencing compound according to the invention from about 0.0001 micromolar to about 10 micromolar. For localized administration, much lower concentrations than this may be effective, and much higher concentrations may be tolerated. Preferably, a total dosage of gene silencing compound according to the invention ranges from about 0.001 mg per patient per day to about 200 mg per kg body weight per day. In certain embodiments, the total dosage may be 0.08, 0.16, 0.32, 0.48, 0.32, 0.64, 1, 10 or 30 mg/kg body weight administered daily, twice weekly or weekly. It may be desirable to administer simultaneously, or sequentially a therapeutically effective amount of one or more of the therapeutic compositions of the invention to an individual as a single treatment episode.

[00176] The methods according to this aspect of the invention are useful for model studies of gene expression. The methods are also useful for the prophylactic or therapeutic treatment of human or animal disease. For example, the methods are useful for pediatric and veterinary inhibition of gene expression applications.

[00177] The examples below are intended to further illustrate certain preferred embodiments of the invention, and are not intended to limit the scope of the invention.

Example 1:

Preparation of Oligonucleotide-based compounds

[00178] The oligonucleotide-based compounds of the invention were chemically synthesized using phosphoramidite chemistry on automated DNA/RNA synthesizer. TAC protected (Except U) 2'-O-TBDMS RNA monomers, A, G, C and U, were purchased from Sigma-Aldrich. 7-deaza-G, inosine and loxoribine monomers were purchased from

ChemGenes Corporation. 0.25M 5-ethylthio-1H-tetrazole, PAC- anhydride Cap A and Cap B were purchased from Glen Research. 3% trichloroacetic acid (TCA) in dichloromethane (DCM) and 5% 3H-1,2-Benzodithiole-3-one-1,1-dioxide (Beaucage reagent) were made in house.

[00179] Oligonucleotide-based compounds of the invention were synthesized at 1-2 μM scale using a standard RNA synthesis protocol.

Cleavage and base deprotection

[00180] Oligonucleotide-based compounds of the invention were cleaved from solid support and the solution was further heated at 65 °C to removing protecting groups of exo cyclic-amines. The resulting solution was dried completely in a SpeedVac.

IE HPLC Purification

[00181] Oligonucleotide-based compounds of the invention were purified by ion exchange HPLC.

Column: Dionex DNAPac 100 column (22X250)

Column Heater: ChromTech TL-105 HPLC column heater, temperature is set to 80 °C.

Buffer A: 20 mM Tris-HCl, pH 7.0, 20% acetinitrile

Buffer B: 3.0 M NaCl, 20 mM Tris-HCl, pH 7.0, 20% acetonitrile

Flow rate: 10ml/min

Gradient:

0-2 min: 0% B

2-11 min: 0% B to 35% B

11-41 min: 35% B to 90% B

41-45 min: 100% B

[00182] Crude solution of oligonucleotide-based compounds of the invention was injected into HPLC. Above gradient is performed and the fractions were collected. All fractions containing more than 90% desired product were mixed, and then the solution was concentrated to almost dry by RotoVap. RNAse-free water was added to make final volume of 10ml.

C-18 Reversed Phase Desalting

[00183] CC-18 Sep-Pak cartridge purchased from Waters was first conditioned with 10ml of acetonitrile followed by 10 ml of 0.5 M sodium acetate. 10 ml of the solution of oligonucleotide-based compounds of the invention was loaded. 15 ml of water was then used to wash out the salt. The oligonucleotide-based compounds of the invention was eluted out by 1 ml of 50% acetonitrile in water.

[00184] The solution is placed in SpeedVac for 30 minutes. The remaining solution was filter through a 0.2 micro filter and then was lyophilized to dryness. The solid was then re-dissolved in water to make the desired concentration.

[00185] The final solution was stored below 0 °C.

Capillary Electrophoresis

[00186] Oligonucleotide-based compounds of the invention were analyzed by capillary electrophoresis according to the following conditions.

Instrument: Beckman 5010

Capillary: 62cm ssDNA capillary

Sample preparation: 0.2 OD of oligonucleotide-based composition according to the invention was dissolved in 200ul of RNAse-free water.

Injection: electro-kinetic injection at 5KV for 5 seconds.

Running condition: 14KV for 50 minutes at 30 °C.

Ion Exchange HPLC analysis

[00187] Oligonucleotide-based compounds of the invention were analyzed by ion exchange HPLC according to the following conditions:

Column: Dionex DNAPac guard column (22X250)

Column Heater: ChromTech TL-105 HPLC column heater, temperature is set to 80 °C.

Buffer A: 100 mM Tris-HCl, pH 8.0, 20% acetinitrile

Buffer B: 2.0 M LiCl, 100 mM Tris-HCl, pH 8.0, 20% acetonitrile

Flow rate: 2ml/min

Gradient:

0-2 min: 0% B

2-10 min: 0% B to 100% B

10-15 min: 100% B

PAGE analysis

[00188] 0.3 OD of oligonucleotide-based compounds of the invention was loaded on 20% polyacrylamide gel and was running at constant power of 4 watts for approximately 5 hours. The gel was viewed under short wavelength UV light.

Dual Luciferase Reporter System Assay

[00189] Hepa 1-6 cells are co-transfected with GSO and target plasmid simultaneously using LIPOFECTAMINE® 2000 on day one (20,000 c/well). RLuc siRNA was used as the positive control and GSO mu/hu universal control was used as the negative control. On day two (24 hours post-transfection), luminescence measurements for both reporter genes are taken separately: Firefly luciferase: expression serves as the normalizer for the assay; Renilla luciferase: substrate includes a "stop" reagent to quench luminescence from firefly. Separate luminescence measurements are taken to correspond to renilla-target transcript expression. Substrate includes DTT to lyse cells. Results are shown in Table 4A and Table 4B.

Table 4A

3GA #	GSO sequence	% KD Luciferase Screen (25nM)
1	3'-GACCGTACACCCAGGCCG-5'-X-5'-GCCGGACCCACATGCCCAG-3'	72.80
2	3'-ACACCCAGGCCGTCCATGG-5'-X-5'-GGTACCTGCCGGACCCACA-3'	74.00
3	3'-TCGACCGTTAGTCCCACCG-5'-X-5'-GCCACCCTGATTGCCAGCT-3'	73.10
4	3'-TCTCCCTCGTTTACGGTGG-5'-X-5'-GGTGGCATTTGCTCCCTCT-3'	62.60
5	3'-TCCCTGCTCTCTTCTGTGG-5'-X-5'-GGTGTCTTCTCTCGTCCCT-3'	20.80
6	3'-ACCCTGCATCCCCGAGTCG-5'-X-5'-GCTGAGCCCCTACGTCCCA-3'	52.80
7	3'-TGTTCCACGTCTCGACCCC-5'-X-5'-CCCCAGCTCTGCACCTTGT-3'	76.50
8	3'-TCCCTTGGTCGTCTCGATC-5'-X-5'-CTAGCTCTGCTGGTTCCCT-3'	71.50
9	3'-TACGTCTAGGGTGTCCGCG-5'-X-5'-GCGCCTGTGGGATCTGCAT-3'	76.32
10	3'-CACGATGTTGACCCGACCG-5'-X-5'-GCCAGCCCAGTTGTAGCAC-3'	80.51
11	3'-TCGTTGGTCTGCCTGTTCG-5'-X-5'-GCTTGTCCGTCTGGTTGCT-3'	64.70
12	3'-TCCCACTGTCTCTCTTCCC-5'-X-5'-CCCTTCTCTCTGTCACCCT-3'	63.00
13	3'-CCCACTGTCTCTCTTCCCG-5'-X-5'-GCCCTTCTCTCTGTCACCC-3'	71.30
14	3'-CCACTGTCTCTCTTCCCGT-5'-X-5'-TGCCCTTCTCTCTGTCACC-3'	61.91
15	3'-ACGATCAGACCCAGGACCG-5'-X-5'-GCCAGGACCCAGACTAGCA-3'	53.22
16	3'-TCAGACCCAGGACCGGCAG-5'-X-5'-GACGGCCAGGACCCAGACT-3'	73.38
17	3'-GTGACTCCGGACTCCTACC-5'-X-5'-CCATCCTCAGGCCTCAGTG-3'	53.02
18	3'-GACTCCGGACTCCTACCTG-5'-X-5'-GTCCATCCTCAGGCCTCAG-3'	51.62
19	3'-CTCCGGACTCCTACCTGTG-5'-X-5'-GTGTCCATCCTCAGGCCTC-3'	56.13
20	3'CCGGACTCCTACCTGTGAC-5'-X-5'-CAGTGTCCATCCTCAGGCC-3'	46.22

21	3'-CCTCTTCGTCCGTCCCACG-5'-X-5'-GCACCCTGCCTGCTTCTCC-3'	68.62
22	3'-TCTTCGTCCGTCCCACGTC-5'-X-5'-CTGCACCCTGCCTGCTTCT-3'	73.88
23	3'-GGGTCCGTCGTCCACAGTG-5'-X-5'-GTGACACCTGCTGCCTGGG-3'	61.92
24	3'-GGATGTCCCTCCCGGTCTA-5'-X-5'-ATCTGGCCCTCCCTGTAGG-3'	40.93
25	3'-ATGTCCCTCCCGGTCTACG-5'-X-5'-GCATCTGGCCCTCCCTGTA-3'	41.82
26	3'-GTCCCTCCCGGTCTACGTC-5'-X-5'-CTGCATCTGGCCCTCCCTG-3'	57.94
27	3'-CCCTCCCGGTCTACGTCAG-5'-X-5'-GACTGCATCTGGCCCTCCC-3'	58.14
28	3'-CTCCCGGTCTACGTCAGTG-5'-X-5'-GTGACTGCATCTGGCCCTC-3'	63.13
29	3'-GGTTCTGACCCGTGTCCTC-5'-X-5'-CTCCTGTGCCCAGTCTTGG-3'	59.63
30	3'-TCGAGGACCGACACCACCC-5'-X-5'-CCCACCACAGCCAGGAGCT-3'	77.64
31	3'-CGAGGACCGACACCACCCG-5'-X-5'-GCCCACCACAGCCAGGAGC-3'	80.53
32	3'-GGGTCCGTCGTGGAGTCCG-5'-X-5'-GCCTGAGGTGCTGCCTGGG-3'	62.62
33	3'-CCGTCCCTTCGACTCCGTC-5'-X-5'-CTGCCTCAGCTTCCCTGCC-3'	73.27
34	3'-CGTCCCTTCGACTCCGTCA-5'-X-5'-ACTGCCTCAGCTTCCCTGC-3'	78.22
35	3'-CCCGTCCGTCTCGACCTCC-5'-X-5'-CCTCCAGCTCTGCCTGCCC-3'	60.58
36	3'-CCGTCCGTCTCGACCTCCG-5'-X-5'-GCCTCCAGCTCTGCCTGCC-3'	54.52
37	3'-CAGGGTTCCAGTCCTCCCG-5'-X-5'-GCCCTCCTGACCTTGGGAC-3'	71.63
38	3'-GGGTTCCAGTCCTCCCGTC-5'-X-5'-CTGCCCTCCTGACCTTGGG-3'	69.94
39	3'-GTTCCAGTCCTCCCGTCCC-5'-X-5'-CCCTGCCCTCCTGACCTTG-3'	71.58
40	3'-GTCCCGGACCCACCCTTCC-5'-X-5'-CCTTCCCACCCAGGCCCTG-3'	57.15
41	3'-CCCGGACCCACCCTTCCAT-5'-X-5'-TACCTTCCCACCCAGGCCC-3'	51.93
42	3'-CGGACCCACCCTTCCATGT-5'-X-5'-TGTACCTTCCCACCCAGGC-3'	31.04
43	3'GACCCACCCTTCCATGTCC-5'-X-5'-CCTGTACCTTCCCACCCAG-3'	44.89
44	3'-GTCCCGGTGGTCGTAGGTC-5'-X-5'-CTGGATGCTGGTGGCCCTG-3'	61.72
45	3'-TCCCGGTGGTCGTAGGTCC-5'-X-5'-CCTGGATGCTGGTGGCCCT-3'	52.12
46	3'TCCGTGGACTCACCGACCC-5'-X-5'-CCCAGCCACTCAGGTGCCT-3'	76.87
47	3'-CTCACCGACCCTGTTCCCT-5'-X-5'-TCCCTTGTCCCAGCCACTC-3'	68.50
48	3'-CACCGACCCTGTTCCCTAG-5'-X-5'-GATCCCTTGTCCCAGCCAC-3'	74.30
49	3'-GCCCTCGACCTGGACGAAC-5'-X-5'-CAAGCAGGTCCAGCTCCCG-3'	67.80
50	3'-TGTCGTTGAAGTCCCCCTC-5'-X-5'-CTCCCCCTGAAGTTGCTGT-3'	76.40
51	3'-TGCCACCACGCCTGATGTT-5'-X-5'-TTGTAGTCCGCACCACCGT-3'	84.40
52	3'-TCCCTTGTGTCTCCAGTGG-5'-X-5'-GGTGACCTCTGTGTTCCCT-3'	58.30
53	3'-ATCCCTCCGTCTCCGTCCG-5'-X-5'-GCCTGCCTCTGCCTCCCTA-3'	76.10
54	3'-GACTTCCTTGTCCGACCCG-5'-X-5'-GCCCAGCCTGTTCCTTCAG-3'	70.30

Where X is glycerol

Table 4B

3GA #	% KD Luciferase Screen (25nM)		3GA #	% KD Luciferase Screen (25nM)
55	72.55		221	64.50
56	16.18		222	73.89
57	68.59		223	81.38

58	82.08
59	64.04
60	61.19
61	55.65
62	29.88
63	44.00
64	73.27
65	69.04
66	76.39
67	67.30
68	84.30
69	61.65
70	59.28
71	60.44
72	49.61
73	65.21
74	52.34
75	82.43
76	68.25
77	83.97
78	82.25
79	67.84
80	41.54
81	80.09
82	53.95
83	74.05
84	2.78
85	53.89
86	53.70
87	34.15
88	77.07
89	23.27
90	41.99
91	41.36
92	60.45
93	58.99
94	74.51
95	10.33
96	9.46
97	42.36
98	27.05
99	24.30
100	10.54

224	70.29
225	69.92
226	81.70
227	59.46
228	81.39
229	88.01
230	75.84
231	58.18
232	29.33
233	61.77
234	72.38
235	45.83
236	39.94
237	66.24
238	49.78
239	23.03
240	59.57
241	41.65
242	44.50
243	18.23
244	37.51
245	58.43
246	70.66
247	74.80
248	70.32
249	90.70
250	73.19
251	81.50
252	87.92
253	76.82
254	55.60
255	42.30
256	44.52
257	81.17
258	64.45
259	79.46
260	41.81
261	46.85
262	83.04
263	78.00
264	69.88
265	59.09
266	39.05

101	85.55
102	60.69
103	63.04
104	59.83
105	57.80
106	71.35
107	74.39
108	74.04
109	80.27
110	89.98
111	86.33
112	88.35
113	84.67
114	57.13
115	56.03
116	77.36
117	72.63
118	74.24
119	79.93
120	86.42
121	57.13
122	49.63
123	65.62
124	72.63
125	96.00
126	71.45
127	-0.08
128	4.49
129	60.37
130	67.01
131	10.42
132	72.11
133	66.46
134	58.22
135	40.56
136	75.82
137	69.22
138	69.06
139	79.03
140	10.17
141	25.50
142	84.19
143	81.61

267	34.97
268	83.20
269	86.16
270	49.03
271	70.17
272	86.40
273	67.96
274	44.65
275	65.68
276	66.68
277	76.67
278	39.46
279	69.63
280	68.44
281	57.77
282	67.85
283	61.74
284	69.87
285	58.11
286	41.07
287	42.40
288	42.09*
289	61.77*
290	33.46*
291	49.08*
292	43.43*
293	40.08*
294	57.06*
295	87.34*
296	76.54*
297	36.96*
298	96.71*
299	70.53*
300	88.21*
301	76.86*
302	85.21*
303	74.25*
304	70.61*
305	83.52*
306	65.18*
307	84.36*
308	91.72
309	93.78

144	70.57
145	78.67
146	66.46
147	58.98
148	69.06
149	97.18
150	81.25
151	30.14
152	73.42
153	79.87
154	63.67
155	71.18
156	64.26
157	84.39
158	91.96
159	87.62
160	85.37
161	90.51
162	90.48
163	88.20
164	82.16
165	79.86
166	88.55
167	91.35
168	81.98
169	94.67
170	84.41
171	82.59
172	31.12
173	67.25
174	36.58
175	55.38
176	73.10
177	77.05
178	91.25
179	83.86
180	85.21
181	72.85
182	61.27
183	81.35
184	68.97
185	67.13
186	57.30

310	86.49
311	79.67
312	78.18
313	68.73
314	49.06
315	51.92
316	64.80
317	49.86
318	60.88
319	54.14
320	54.27
321	64.20
322	57.54
323	64.73
324	24.73
325	-3.86
326	58.29
327	85.58
328	38.27
329	54.35
330	37.69
331	42.71
332	77.86
333	34.95
334	29.20
335	41.76
336	55.25
337	56.23
338	44.34
339	42.26
340	33.54
341	32.88
342	46.91
343	25.39
344	53.54
345	68.08
346	65.26
347	70.49
348	33.62
349	78.29
350	87.30
351	92.56
352	82.30

187	79.41	353	62.64
188	41.38	354	84.10
189	50.80	355	72.48
190	52.18	356	87.25
191	63.14	357	68.93
192	82.52	358	77.23
193	41.99	359	74.70
194	73.39	360	43.71
195	76.17	361	86.31
196	85.66	362	52.57
197	98.60	363	38.62
198	47.11	364	64.49
199	42.47	365	66.70
200	87.17	366	77.24
201	74.36	367	89.81
202	58.24	368	77.82
203	59.21	369	62.31
204	42.36	370	80.21
205	74.17	371	58.76
206	76.54	372	71.34
207	30.41	373	65.23
208	68.55	374	65.58
209	69.73	375	78.67
210	59.73	376	67.01
211	54.92	377	32.15
212	56.90	378	49.07
213	69.09	379	53.07
214	77.40	380	58.24
215	39.73	381	72.09
216	39.23	382	63.90
217	41.13	383	68.54
218	23.48	384	15.69
219	79.92	385	25.43
220	29.57	386	40.49

For 3GA compounds numbers 55 through 386 listed in Table 4B, glycerol is the non-nucleotidic linker.

Flow cytometric analysis

[00190] Whole blood samples with anticoagulant EDTA from mice in study were stained for 30 minutes in the dark at room temperature with the following labeled antibodies

from BD Biosciences in the presence of mouse Fc blocker (Affymetrix eBioscience, 14-0161): rat anti-mouse CD3-Alexa Fluor 647 (557869), rat anti-mouse CD4-Alexa Fluor 647 (557681), rat anti-mouse CD8-Alexa Fluor 488 (557668) or the corresponding isotype controls. Red blood cells were lysed with freshly prepared 1x RBC lysis buffer (eBioscience, 00-4300) and washed with flow cytometry staining buffer (BD Biosciences, 554657). Resuspended cell suspensions in the flow cytometry staining buffer were run on BD Accuri C6 to acquire data and analyzed by FLOWJO (TreeStar).

IC50 Analysis

[00191] Hepa 1-6 cells are co-transfected with 3GA and target plasmid simultaneously using LIPOFECTAMINE® 2000 on day one (20,000 c/well). Concentration of 3GAs were ranging from 0.019 to 41.7 nM with a 3-fold increment. RLuc siRNA was used as the positive control and 3GA mu/hu universal control was used as the negative control. On day two (24 hours post-transfection), luminescence measurements for both reporter genes are taken separately: Firefly luciferase: expression serves as the normalizer for the assay; Renilla luciferase: substrate includes a "stop" reagent to quench luminescence from firefly. Separate luminescence measurements are taken to correspond to renilla-target transcript expression. Substrate includes DTT to lyse cells. IC50 of 3GAs was calculated using GraphPad Prism 6. Results are shown in Table 5.

Table 5

3GA #	target	Target Site	GSO Sequence 5' to 3'	IC_{50} (nM)
75	mIDO1	138	CTAGCCACAAGGACCCAGG	33.1
81	hIDO1	172	GCAGAGCAAAGCCCACTTC	3.49
92		1239	GCCTCCAGTTCCTTTGGCT	1.53
3	mPD1	115	GCCACCCTGATTGCCAGCT	59.0
10	hPD1	108	GCCAGCCCAGTTGTAGCAC	3.87
33		1591	CTGCCTCAGCTTCCCTGCC	1.57
46		2024	CCCAGCCACTCAGGTGCCT	3.16
54	mPD-L1	3311	GCCCAGCCTGTTCCTTCAG	14.4
55	hPD-L1	571	GGTAGCCCTCAGCCTGACA	5.00
58		1080	CCCACATTGCCTGCATCCC	2.42
64		1855	TCTGTTTGCTTCCTCAGCT	2.51
158	mTIM3	1425	ACTTATCACCCTCCTGCCT	5.55
169	hTIM3	59	CTGCCAGGTCTACAGTCAC	13.8

180		1506	CAGCACCCAGTTTTCCCTA	6.10
183		1716	ATCCCAGCCACTCAGGAGG	32.7
110	mLAG3	1108	GCTCCAGACCCAGAACCTT	6.49
124	hLAG3	1356/1369	GACAGTGGCATTGAGCTGC	11.9
122		1822/1841	GCTCCAGCTCCTCTATCTT	9.10
143		1848	GGTTCTTGCTCCAGCTCCT	5.03
195	mCTLA4	563	GTTGCCCATGCCCACAAAG	19.6
225	hCTLA4	1822	GCCTTCTTCTGTCCATGGC	1.71
247	mIDO2	2748	GGTGACTTCCAGGTCTGCA	0.247
249	hIDO2	156	GGTGTCCATTGCCTTCTGT	2.73
259		1137	AGTCACCACTTTCCTTGCT	3.36
262		1363	GGCTTCCCATGCTTTGCCT	1.28
361	mOX40L	1069	TGGACACCACCCTTTCCAT	0.673
368	hOX40L	712	TGGTCCGTGGTCGTACTTG	0.553
370		1104	CTCCTCGTTCCTCACTTCC	5.01

In Vivo Mouse Tumor Model

[00192] Colon tumor can be implanted in BALB/c mice by subcutaneous injection of 10^6 CT26.WT cells at right flank (Tumor 1) and 10^6 CT26.CL25 cells at left flank (Tumor 2) on day 0. Treatment can be initiated on day 6 or when tumor size reached to 70 to 80 mm^3 by intra-tumor injection of gene silencing compound according to the invention at various dosages (e.g., 2 mg/kg, 5 mg/kg, 12.5 mg/kg, or 25 mg/kg) on day 6, 10, 13, 16, 20, and 22.

[00193] Tumor growth can be monitored twice per week throughout the study period. The study can be terminated with blood, spleen and tumor tissues collected for further evaluation. T lymphocyte population in blood and spleen samples were detected and analyzed by flow cytometry. Spleen IFN-γ-producing cells were detected with ELISPOT assay after culture of spleen cells for 24 hours with tumor antigen beta-gal or AH1 peptide. Tumor tissues were analyzed for gene expression by RT-PCR.

EQUIVALENTS

[00194] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. For example, antisense oligonucleotides that overlap with the oligonucleotides may be used. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

CLAIMS

What is claimed is:

1. A 3rd generation antisense (3GA) compound comprising two oligonucleotides linked at their 5' ends, each oligonucleotide, independently, consists of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389, SEQ ID NO: 390, SEQ ID NO: 391, SEQ ID NO: 392, SEQ ID NO: 393, SEQ ID NO: 394, SEQ ID NO: 395, SEQ ID NO: 396, SEQ ID NO: 397, SEQ ID NO: 398, SEQ ID NO: 399, SEQ ID NO: 400, SEQ ID NO: 401, SEQ ID NO: 402, SEQ ID NO: 403, SEQ ID NO: 404, SEQ ID NO: 405, or SEQ ID NO: 406.

2. The 3GA compound according to claim 1, wherein each oligonucleotide, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 388, SEQ ID NO: 390, SEQ ID NO: 392, SEQ ID NO: 394, SEQ ID NO: 396, SEQ ID NO: 398, SEQ ID NO: 400, SEQ ID NO: 402, SEQ ID NO: 404, or SEQ ID NO: 406.

3. The 3GA compound according to claim 1, wherein the nucleobase sequence of each oligonucleotide is, independently, at least 90% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, or 406.

4. The 3GA compound according to claim 3, wherein the nucleobase sequence of each oligonucleotide is, independently, at least 90% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 388, 390, 392, 394, 396, 398, 400, 402, 404, or 406.

5. The 3GA compound according to claim 1, wherein each oligonucleotide, independently, comprises a portion of at least 12 contiguous nucleobases of 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, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 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, 138,

139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, or combinations thereof.

6. The 3GA compound according to claim 5, wherein each oligonucleotide, independently, comprises a portion of least 12 contiguous nucleobases of SEQ ID NOs: 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, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 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, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318,

319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, and is at least 80% complimentary to its target site with SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389, SEQ ID NO: 390, SEQ ID NO: 391, SEQ ID NO: 392, SEQ ID NO: 393, SEQ ID NO: 394, SEQ ID NO: 395, SEQ ID NO: 396, SEQ ID NO: 397, SEQ ID NO: 398, SEQ ID NO: 399, SEQ ID NO: 400, SEQ ID NO: 401, SEQ ID NO: 402, SEQ ID NO: 403, SEQ ID NO: 404, SEQ ID NO: 405, or SEQ ID NO: 406.

7. The 3GA compound according to claim 6, wherein each oligonucleotide, independently, comprises a portion of at least 12 contiguous nucleobases of SEQ ID NOs: 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386, and is at least 80% complimentary to its target site within SEQ ID NO: 388, SEQ ID NO: 390, SEQ ID NO: 392, SEQ ID NO: 394, SEQ ID NO: 396, SEQ ID NO: 398, SEQ ID NO: 400, SEQ ID NO: 402, SEQ ID NO: 404, or SEQ ID NO: 406.

8. A composition comprising a 3GA compound according to claim 1 and a pharmaceutically acceptable carrier.

9. The composition according to claim 8, further one or more vaccines, antigens, antibodies, cytotoxic agents, chemotherapeutic agents, kinase inhibitors, allergens, antibiotics, agonist, antagonist, antisense oligonucleotides, ribozymes, RNAi molecules, siRNA molecules, miRNA molecules, aptamers, proteins, gene therapy vectors, DNA vaccines, adjuvants, co-stimulatory molecules or combinations thereof.

10. A method for inhibiting PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L mRNA or protein expression, the method comprising contacting a cell with at least one 3GA compound according to claim 1.

11. The method according to claim 10, wherein the cell is contacted with two or more 3GA compounds targeting different regions of the same checkpoint.

12. The method according to claim 10, wherein the cell is contacted with two or more 3GA compounds targeting different checkpoints.

13. A method for inhibiting immune system tolerance to tumors comprising administering to an animal at least one 3GA compound according to claim 1 or composition according to claim 8 to reduce PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, and/or OX40L mRNA or protein expression.

14. A method for the treatment of a disease, disorder, or condition associated with PD1, PDL1, IDO1, LAG3, TIM3, CTLA4, IDO2, CEACAM1, OX40, or OX40L in an individual in need thereof, the method comprising administering at least one 3GA compound according to claim 1.

15. The method according to claim 1, wherein the disease, disorder, or condition is a hyperproliferative disease or an autoimmune disease.

16. The method according to claim 2, wherein the hyperproliferative disease is cancer, carcinomas, sarcomas, lymphomas, leukemias and associated malignancies and metastases.

17. The method according to claim 3, wherein the 3GA compound is administered intratumorally.