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(54) Title: MICRORNA-34 IMMUNOTHERAPY

(57) **Abstract**: A method of treating a PD-L1 expressing cancer in an individual in need thereof by administering a synthetic miR-34 oligonucleotide or a combination of a synthetic miR-34 oligonucleotide and a PD-L1 or PD-1 inhibitor. A method of treating a chronic infectious disease in an individual in need thereof by administering a synthetic miR-34 oligonucleotide and a PD-L1 or PD-1 inhibitor.

MICRORNA-34 IMMUNOTHERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Applications No. 62/142,790, filed on April 3, 2015, and U.S. Provisional Application 62/293,497, filed February 10, 2016, which are incorporated herein by reference in their entirety.

SUMMARY OF THE INVENTION

[0002] Disclosed herein, in certain embodiments, are methods of treating a PD-L1 expressing cancer in an individual in need thereof comprising: (a) identifying the cancer as a PD-L1 expressing cancer; and (b) providing a synthetic miR-34 oligonucleotide to the individual. In some embodiments, the methods further comprise providing a PD-1 or PD-L1 inhibitor to the individual. In some embodiments, the PD-1 or PD-L1 inhibitor is an antibody, small interfering RNA, or antisense RNA. In some embodiments, the synthetic miR-34 oligonucleotide and the PD-1 or PD-L1 inhibitor are synergistic. In some embodiments, the synthetic miR-34 oligonucleotide is provided before the PD-1 or PD-L1 inhibitor. In some embodiments, the methods further comprise providing radiotherapy to the individual. In some embodiments, the synthetic miR-34 oligonucleotide is provided before the radiotherapy. In some embodiments, the cancer is a solid tumor. In some embodiments, the solid cancer is a melanoma, nasopharyngeal cancer, neuroendrocrine tumor, lung cancer, colon cancer, urothelial cancer, bladder cancer, liver cancer, multiple myeloma, ovarian cancer, gastric carcinoma, esophageal cancer, pancreatic cancer, kidney cancer, breast cancer, or lymphoma. In some embodiments, the lung cancer is a non-small cell lung cancer (NSCLC). In some embodiments, the lung cancer is a small-cell lung cancer (SCLC). In some embodiments, the liver cancer is a hepatocellular carcinoma (HCC). In some embodiments, the cancer is a leukemia. In some embodiments, the cancer is a lymphoma. In some embodiments, the cancer as a PD-L1 expressing cancer comprises measuring the PD-L1 expression in a cancer cell from the individual and comparing to a control. In some embodiments, the PD-L1 expression is overexpressed compared to the control. In some embodiments, the methods further comprise selecting the individual having functional p53. In some embodiments, the synthetic miR-34 oligonucleotide is administered in a liposomal formulation. In some embodiments, T cell exhaustion is prevented or reduced. In some embodiments, the synthetic miR-34 oligonucleotide comprises: (a) an active strand comprising a sequence at least 80% identical to a mature miRNA; and (b) a separate passenger strand that is at least 60% complementary to the active strand. In some

embodiments, the passenger strand of the synthetic miR-34 oligonucleotide comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine. In some embodiments, the 5' terminal cap is NH_2-$(CH_2)_6$-O-. In some embodiments, the mature miRNA comprises: miR-34a (SEQ ID NO:1), miR-34b (SEQ ID NO:2), miR-34c (SEQ ID NO:3), or a miR-34 consensus sequence (SEQ ID NO:4). In some embodiments, the mature miRNA comprises: miR-449a (SEQ ID NO:5), miR-449b (SEQ ID NO:6), miR-449c (SEQ ID NO:7), or a miR-449 consensus sequence (SEQ ID NO:8). In some embodiments, the mature miRNA comprises a miR-34/449 seed sequence (SEQ ID NO:9).

[0003] Disclosed herein, in certain embodiments, are methods of treating a PD-L1 expressing cancer in an individual in need thereof comprising: (a) providing a synthetic miR-34 oligonucleotide to the individual; and (b) providing a PD-1 or PD-L1 inhibitor to the individual. In some embodiments, the PD-1 or PD-L1 inhibitor is an antibody, small interfering RNA, or antisense RNA. In some embodiments, the synthetic miR-34 oligonucleotide and the PD-1 or PD-L1 inhibitor are synergistic. In some embodiments, the synthetic miR-34 oligonucleotide is provided before the PD-1 or PD-L1 inhibitor. In some embodiments, the methods further comprise providing radiotherapy to the individual. In some embodiments, the synthetic miR-34 oligonucleotide is provided before the radiotherapy. In some embodiments, the cancer is a solid tumor. In some embodiments, the solid cancer is a melanoma, nasopharyngeal cancer, neuroendrocrine tumor, lung cancer, colon cancer, urothelial cancer, bladder cancer, liver cancer, multiple myeloma, ovarian cancer, gastric carcinoma, esophageal cancer, pancreatic cancer, kidney cancer, breast cancer, or lymphoma. In some embodiments, the lung cancer is a non-small cell lung cancer (NSCLC). In some embodiments, the lung cancer is a small-cell lung cancer (SCLC). In some embodiments, the liver cancer is a hepatocellular carcinoma (HCC). In some embodiments, the cancer is a leukemia. In some embodiments, the cancer is a lymphoma. In some embodiments, identifying the cancer as a PD-L1 expressing cancer comprises measuring the PD-L1 expression in a cancer cell from the individual and comparing to a control. In some embodiments, the PD-L1 expression is overexpressed compared to the control. In some embodiments, the methods further comprise selecting the individual having functional p53. In some embodiments, the synthetic miR-34 oligonucleotide is administered in a liposomal formulation. In some embodiments, T cell exhaustion is prevented or reduced. In some embodiments, the synthetic miR-34 oligonucleotide comprises: (a) an active strand comprising a sequence at least 80% identical to a mature miRNA; and (b) a separate passenger strand that is at least 60% complementary to the active strand. In some embodiments, the passenger strand of the synthetic miR-34 oligonucleotide comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine. In some embodiments, the 5'

terminal cap is NH_2-$(CH_2)_6$-O-. In some embodiments, the mature miRNA comprises: miR-34a (SEQ ID NO:1), miR-34b (SEQ ID NO:2), miR-34c (SEQ ID NO:3), or a miR-34 consensus sequence (SEQ ID NO:4). In some embodiments, the mature miRNA comprises: miR-449a (SEQ ID NO:5), miR-449b (SEQ ID NO:6), miR-449c (SEQ ID NO:7), or a miR-449 consensus sequence (SEQ ID NO:8). In some embodiments, the mature miRNA comprises a miR-34/449 seed sequence (SEQ ID NO:9).

[0004] Disclosed herein, in certain embodiments, are methods of treating an individual suffering from a chronic infectious disease comprising: (a) providing a synthetic miR-34 oligonucleotide to the individual; and (b) providing a PD-1 or PD-L1 inhibitor to the individual. In some embodiments, the PD-1 or PD-L1 inhibitor is an antibody, small interfering RNA, or antisense RNA. In some embodiments, the synthetic miR-34 oligonucleotide and the PD-1 or PD-L1 inhibitor are synergistic. In some embodiments, the synthetic miR-34 oligonucleotide is provided before the PD-1 or PD-L1 inhibitor. In some embodiments, the infectious disease is a persistent viral infection. In some embodiments, the viral infection is human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), rhinovirus (common cold), herpes simplex virus (HSV), or respiratory syncytial virus (RSV). In some embodiments, the infectious disease is a persistent bacterial infection. In some embodiments, the bacterial infection is *Helicobacter pylori*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, or *Chlamydia trachomatis*. In some embodiments, the infectious disease is the result of infection by a parasite, protozoan, or metazoan. In some embodiments, the parasite, protozoan, or metazoan is *Schistosoma mansoni*, *Taenia crassiceps*, or *Leishmania mexicana*. In some embodiments, the methods further comprise selecting the individual having functional p53. In some embodiments, the synthetic miR-34 oligonucleotide is administered in a liposomal formulation. In some embodiments, T cell exhaustion is prevented or reduced. In some embodiments, the synthetic miR-34 oligonucleotide comprises: (a) an active strand comprising a sequence at least 80% identical to a mature miRNA; and (b) a separate passenger strand that is at least 60% complementary to the active strand. In some embodiments, the passenger strand of the synthetic miR-34 oligonucleotide comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine. In some embodiments, the 5' terminal cap is NH_2-$(CH_2)_6$-O-. In some embodiments, the mature miRNA comprises: miR-34a (SEQ ID NO:1), miR-34b (SEQ ID NO:2), miR-34c (SEQ ID NO:3), or a miR-34 consensus sequence (SEQ ID NO:4). In some embodiments, the mature miRNA comprises: miR-449a (SEQ ID NO:5), miR-449b (SEQ ID NO:6), miR-449c (SEQ ID NO:7), or a miR-449 consensus sequence (SEQ ID NO:8). In some embodiments, the mature miRNA comprises a miR-34/449 seed sequence (SEQ ID NO:9).

[0005] Disclosed herein, in certain embodiments, are compositions comprising: (a) a synthetic miR-34 oligonucleotide, comprising: (i) an active strand comprising a sequence at least 80% identical to a mature miRNA; and (ii) a separate passenger strand that is at least 60% complementary to the active strand; and (b) a PD-1 or PD-L1 inhibitor. In some embodiments, the passenger strand of the synthetic miR-34 oligonucleotide comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine. In some embodiments, the 5' terminal cap is NH_2-$(CH_2)_6$-O-. In some embodiments, the mature miRNA comprises: miR-34a (SEQ ID NO:1), miR-34b (SEQ ID NO:2), miR-34c (SEQ ID NO:3), or a miR-34 consensus sequence (SEQ ID NO:4). In some embodiments, the mature miRNA comprises: miR-449a (SEQ ID NO:5), miR-449b (SEQ ID NO:6), miR-449c (SEQ ID NO:7), or a miR-449 consensus sequence (SEQ ID NO:8). In some embodiments, the mature miRNA comprises a miR-34/449 seed sequence (SEQ ID NO:9). In some embodiments, the PD-1 or PD-L1 inhibitor is an antibody, small interfering RNA, or antisense RNA. In some embodiments, the synthetic miR-34 oligonucleotide and the PD-1 or PD-L1 inhibitor are synergistic. In some embodiments, the synthetic miR-34 oligonucleotide is provided before the PD-1 or PD-L1 inhibitor.

INCORPORATION BY REFERENCE

[0006] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0008] **FIGS. 1A-G** illustrate p53 regulation of PD-L1 via miR-34. **FIG. 1A** illustrates upregulation of miR-34a, miR-34b, and miR-34c in HCT116 p53+/+ cells treated with nutlin 3 (10 μM for 24 h) ($P = 0.0001$, $P < 0.001$, $P < 0.001$).**FIG. 1B** illustrates downregulation of PD-L1 expression in HCT116 p53+/+ relative to p53-/- cells. **FIG. 1C** illustrates H1299 p53-inducible cells treated with ponasterone A (PoA) compared with their respective controls, confirming that p53 stabilization induces miR-34a, miR-34b, and miR-34c expression ($P=0.001$, $P=0.004$, $P=0.09$). **FIG. 1D** illustrates downregulation of PD-L1 in H1299 p53-inducible cells. **FIG. 1E** illustrates downregulation of miR-34a, but not miR-34b or miR-34c, in H460 cells expressing p53-targeting shRNA compared with control ($P = 0.006$, $P=0.19$, $P=0.20$). **FIG. 1F** illustrates upregulation of

PD-L1 in H460 cells treated with p53-shRNA. **FIG. 1G** illustrates immunohistochemical staining in samples from patients with NSCLC showing higher PD-L1 protein levels (top row) in tumors with p53 mutation than in tumors with wild-type (wt) p53 (3 patients per group)as well as chromogenic in situ hybridization staining (CISH; bottom row) indicating downregulation of miR-34a in tumors with mutated p53 relative to tumors with wt p53.

[0009] **FIGS. 2A-F** illustrate p53 expression is inversely correlated with PD-L1 in patients with non-small cell lung cancer (NSCLC). **FIG. 2A** illustrates correlation between p53 and PD-L1 (CD274) mRNA expression in samples from 181 patients with NSCLC from The Cancer Genome Atlas (TCGA) ($P < 0.001$). **FIG. 2B** illustrates PD-L1 expression in patients with p53-mutated tumors (n=84) and p53 wild-type (wt) tumors (n=97) showed that tumors with mutated p53 had higher PD-L1 levels than did those with wt p53 ($P = 0.0294$). **FIG. 2C** illustrates miR-34a levels were lower in tumors with mutated p53 versus wt p53 ($P = 0.0103$). **FIG. 2D** illustrates a Kaplan-Meier overall survival curve showing that patients with NSCLC that expressed high PD-L1 and low p53 levels had lower survival rates than did patients with low PD-L1/high p53 tumors ($P = 0.0053$). **FIG. 2E** illustrates a Kaplan-Meier overall survival curve showing that patients with high miR-34a/high p53 or high p53 only had better survival rates than did patients with low miR-34a/low p53 ($P = 0.0053$). **FIG. 2F** illustrates a Kaplan-Meier overall survival curve showing that patients with high miR-34a/high p53 or high p53 only had better survival rates than did patients with low p53 only ($P = 0.03$).

[0010] **FIGS. 3A-L** illustrate regulation of PD-L1 by miR-34s. **FIG. 3A** illustrates PD-L1 expression in A549, H460, and H1200 cells transfected with miR-34a, miR-34b, and miR-34c. **FIG. 3B** illustrates the transfection efficiency of A549 cells 24 hours after treatment with 100 nM of miR-34a compared with a scrambled control. **FIG. 3C** illustrates the transfection efficiency of H460 cells 24 hours after treatment with 100 nM of miR-34a compared with a scrambled control. **FIG. 3D** illustrates the transfection efficiency of H1299 cells 24 hours after treatment with 100 nM of miR-34a compared with a scrambled control. **FIG. 3E** exemplifies a western blot showing PD-L1 expression in A549 cells 96 hours after transfection with miR-34a compared. **FIG. 3F** exemplifies a western blot showing PD-L1 expression in H460 cells 96 hours after transfection with miR-34a. **FIG. 3G** exemplifies a western blot showing PD-L1 expression in H1299 cells 96 hours after transfection with miR-34a. **FIG. 3H** illustrates the transfection efficiency of A549 cells 24 hours after treatment with 100nm of miR-34b compared with a scrambled control. **FIG. 3I** illustrates the transfection efficiency of A549 cells 24 hours after treatment with 100nm of miR-34c compared with a scrambled control. **FIG. 3J** exemplifies a western blot showing PD-L1 expression in A549 cells 96 hours after transfection with miR-34b or miR-34c. **FIG. 3K** illustrates luciferase

activity in cells cotransfected with miR-34a, miR-34b, miR-34c, or a scrambled control and a luciferase reporter construct encoding the luciferase gene fused either to the wild-type PD-L1 3' UTR (PDL1 wt) or a mutated PD-L1 3' UTR (PDL1 mut). **FIG. 3L** exemplifies predicted binding site of miR-34a in the 3' untranslated region (UTR) of PD-L1. Underlined residues indicate bases that have been changed by site-directed mutagenesis.

[0011] **FIGS. 4A-H** illustrate therapeutic delivery of miR-34a represses PD-L1 *in vivo*. **FIG. 4A** illustrates miR-34a expression in 344SQ tumors treated with MRX34 by quantitative polymerase chain reaction (qPCR). **FIG. 4B** illustrates PD-L1 expression in 344SQ tumors treated with MRX34 by flow cytometry. **FIG. 4C** exemplifies a western blot showing PD-L1 expression in 344SQ tumors treated with MRX34. **FIG. 4D** illustrates PD-L1 expression percentage, as determined from flow cytometry in 344SQ tumors treated with MRX34. **FIG. 4E** illustrates immunohistochemical staining of 344SQ tumor tissue. **FIG. 4F** illustrates PD-L1 immunohistochemical staining (IHC) scores of the 344SQ tumors treated with MRX34. **FIG. 4G** illustrates immunohistochemical staining of subcutaneous H1299 NSCLC xenografts. **FIG. 4H** illustrates PD-L1 IHC scores of H1299 NSCLC xenografts treated with MRX34.

[0012] **FIGS. 5A-W** illustrate that therapeutic miR-34a delivery combined with radiation (RT) increases tumor-infiltrating CD8+ T cells and decreases PD-1+ T cells, macrophages, and T-regulatory cells one week after treatment completion. **FIG. 5A** illustrates MRX34 + RT increased the number of CD8+ cells compared with control or either treatment given alone (P = 0.004, n=4) one week after treatment, as determined by flow cytometry. **FIG. 5B** illustrates a contour plot of control CD8+ cell data after one week. **FIG. 5C** illustrates a contour plot of CD8+ cell data one week after administration of MRX34. **FIG. 5D** illustrates a contour plot of CD8+ cell data one week after administration of radiotherapy. **FIG. 5E** illustrates a contour plot of CD8+ cell data one week after administration of MRX34 and radiotherapy. **FIG. 5F** illustrates MRX34 significantly reduced the numbers of PD-1+ T cells (P=0.001, n=4). MRX34+RT combination treatment was significantly more efficient in reducing the numbers of PD-1+ T cells (P = 0.02, n=4) and macrophages than RT alone. **FIG. 5G** illustrates a contour plot of control PD-1+ T cell data after one week. **FIG. 5H** illustrates a contour plot of PD-1+ T cell data one week after administration of MRX34. **FIG. 5I** illustrates a contour plot of PD-1+ T cell one week after administration of radiotherapy. **FIG. 5J** illustrates a contour plot of PD-1+ T cell one week after administration of MRX34 and radiotherapy. **FIG. 5K** illustrates MRX34 significantly reduced the numbers of macrophages (P = 0.008, n=4). **FIG. 5L** illustrates a contour plot of control macrophage data after one week. **FIG. 5M** illustrates a contour plot of macrophage data one week after administration of MRX34. **FIG. 5N** illustrates a contour plot of macrophage one week after administration of

radiotherapy. **FIG. 5O** illustrates a contour plot of macrophage one week after administration of MRX34 and radiotherapy. **FIG. 5P** illustrates RT increased the numbers of T regulatory cells (Tregs) over the control condition, but none of the treatments showed significant effects on Tregs. **FIG. 5Q** illustrates a contour plot of control T –regulatory (Treg) cell data after one week. **FIG. 5R** illustrates a contour plot of Treg cell data one week after administration of MRX34. **FIG. 5S** illustrates a contour plot of Treg cell one week after administration of radiotherapy. **FIG. 5T** illustrates a contour plot of Treg cell one week after administration of MRX34 and radiotherapy. **FIG. 5U illustrates interferon gamma (IFNγ) levels were increased by MRX34 only and MRX34 +** RT versus control or RT alone. **FIG. 5V** illustrates MRX34 + RT increased levels of tumor **necrosis factor alpha (TNFα). FIG. 5W** illustrates MRX34 + RT delayed tumor growth compared with the control condition in a 344SQ mouse model.

DETAILED DESCRIPTION OF THE INVENTION

[0013] Disclosed herein, in certain embodiments, are methods of treating a PD-L1 expressing cancer in an individual in need thereof comprising: (a) identifying the cancer as a PD-L1 expressing cancer; and (b) providing a synthetic miR-34 oligonucleotide to the individual.

[0014] Disclosed herein, in certain embodiments, are methods of treating a PD-L1 expressing cancer in an individual in need thereof comprising: (a) providing a synthetic miR-34 oligonucleotide to the individual; and (b) providing a PD-1 or PD-L1 inhibitor to the individual.

[0015] Disclosed herein, in certain embodiments, are methods of treating an individual suffering from a chronic infectious disease comprising: (a) providing a synthetic miR-34 oligonucleotide to the individual; and (b) providing a PD-1 or PD-L1 inhibitor to the individual.

[0016] Disclosed herein, in certain embodiments, are compositions comprising: (a) a synthetic miR-34 oligonucleotide, comprising: (i) an active strand comprising a sequence at least 80% identical to a mature miRNA; and (ii) a separate passenger strand that is at least 60% complementary to the active strand; and (b) a PD-1 or PD-L1 inhibitor.

Definitions

[0017] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including”, “includes”, “having”, “has”, “with”, or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising.”

[0018] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

[0019] The terms "subject," "individual," and "patient" are used interchangeably herein to refer to a vertebrate, for example, a mammal. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. Tissues, cells, and their progeny of a biological entity obtained in vivo or cultured in vitro are also encompassed. Designation as a "subject" does not necessarily entail supervision of a medical professional.

[0020] "Treating" or "treatment" of a state, disorder or condition (*e.g.*, cancer) includes: (1) preventing or delaying the appearance of clinical or sub-clinical symptoms of the disorder developing in a human that is afflicted with or pre-disposed to the disorder but does not yet experience or display clinical or subclinical symptoms of the disorder; and/or (2) inhibiting the disorder, including arresting, reducing or delaying the clinical manifestation of the disorder or at least one clinical or sub-clinical symptom thereof; and/or (3) relieving the disorder, *e.g.*, causing regression of the disorder or at least one of its clinical or sub-clinical symptoms; and/or (4) causing a decrease in the severity of one or more symptoms of the disorder. The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician.

Inhibitory Regulators and T Cell Exhaustion

[0021] Inhibitory receptors (iRs) are co-inhibitory molecules that negatively interfere with T cell activation and function. One of the most well-studied T cell-related iRs is programmed cell death 1 (PDCD1, PD1, PD-1), which is induced by T cell receptor or B cell receptor signaling and remains high under persistent antigen stimulation. PD-1, along with its ligands, PD-L1 and PD-L2 (and wherein PD-1:PD-L refers to the pathway interaction between receptor and ligand), contributes to inducing and maintaining peripheral tolerance. Proper functioning of the PD-1: PD-L pathway is therefore essential to thwart self-reactive T cells and protect against autoimmunity.

[0022] In some instances, persistent antigen stimulation and inflammation due to chronic infection leads to a loss of function in CD8 T cells, termed T cell exhaustion. Exhausted T cells lose robust

effector functions, upregulate multiple inhibitory receptors (including PD-1), and are defined by an altered transcriptional activity. Thus, immunotherapeutic strategies focusing on blocking iRs in order to restore T cell function ("checkpoint blockade") are promising.

[0023] In some embodiments, T cell exhaustion occurs due to chronic viral, bacterial, or parasitic infections. In some embodiments, T cell exhaustion occurs due to cancer. In some embodiments, blocking or otherwise disrupting the PD-1:PD-L pathway reverses, reduces, or suppresses T cell exhaustion.

Cancer

[0024] In some embodiments, the methods are applicable to the treatment of cancer, including cancer in an individual or *in vitro* treatment of isolated cancer cells. In some embodiments, the cancer is relapsed or refractory. In some embodiments, the cancer has metastasized. In some embodiments, the cancer is a solid tumor. In some embodiments, the solid tumor is a melanoma, neuroendocrine tumor, lung cancer, bladder cancer, glioblastoma, colon cancer, liver cancer, melanoma, multiple myeloma, ovarian cancer, kidney cancer, head and neck cancers, esophageal cancer, gastric carcinoma, pancreatic cancer, or breast cancer.

[0025] In some embodiments, the solid tumor is a lung cancer. In some embodiments, the lung cancer is a non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC).

[0026] In some embodiments, the solid tumor is liver cancer. In some embodiments, the liver cancer is a hepatocellular carcinoma (HCC).

[0027] In some embodiments, the cancer is a hematological malignancy. In some embodiments, the hematological malignancy is a leukemia or a lymphoma. In some embodiments, the leukemia is chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS). In some embodiments, the lymphoma is a T-cell lymphoma, multiple myeloma, or B-cell lymphoma (BCL). In some embodiments, the B-cell lymphoma is a large diffuse B-cell lymphoma (DLBCL).

[0028] In some embodiments, the cancer is a PD-L1 (e.g., PDL1, PD-L1, CD274, B7H, B7-H, B7H1, B7-H1) expressing cancer. In some embodiments, where the methods target PD-L1 expressing cancer, a method includes selecting the individual having PD-L1 overexpression. In some embodiments, any suitable diagnostic assays are used to detect biomarkers of PD-L1 overexpression. In some embodiments, an immunological assay (immunohistochemistry, IHC; antibody based cell sorting) is adapted for use with tissues, for example tumor tissue and/or immune cells (e.g., whether they are tumor infiltrating immune cells or circulating immune cells). In some embodiments, tissue is collected in a way that is applicable to histology (e.g., FFPE, fresh frozen or smears or cell suspension) and used in immunohistochemistry stainings (e.g., usually

chromogenic, but also fluorescent) or antibody based cell sorting. In some embodiments, quantitative PCR methods, as well as FISH (fluorescent in situ hybridization), and CISH (chromogenic ISH) are used for DNA mutations (e.g., such as gene recombinations, deletions, amplifications, mutations).

[0029] In some embodiments, the cancer is a p53 expressing cancer. In some embodiments, where the methods of the invention target p53 expressing cancer, the method includes selecting the individual having p53 overexpression. In some embodiments, the method includes selecting an individual having functional p53. In some embodiments, known diagnostic assays are used to detect biomarkers. In some embodiments, an immunological assay (IHC) on tissues using a DO7 clone antibody is suitable to detect overexpressed nuclear staining of p53 which indicates mutated p53. In some embodiments, quantitative PCR methods, as well as FISH (fluorescent in situ hybridization), and CISH (chromogenic ISH) are applied.

[0030] In some embodiments, the cancer is genotyped. In some embodiments, any suitable means of assaying genotype is contemplated for use with the method disclosed herein.

[0031] In some instances, T cell exhaustion occurs during cancer. In some instances, blocking the PD-1:PD-L inhibitory receptor pathway reinvigorates CD8+ T cell responses. In some embodiments, the synthetic miR-34 oligonucleotide reverses, reduces, or suppresses T cell exhaustion.

Infectious Disease

[0032] In some embodiments, these methods are applicable to the treatment of infectious disease. In some embodiments, the infectious disease is a chronic or persistent viral infection, bacterial infection, or parasitic, protozoan, or metazoan infection. In some embodiments, the viral infection is human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCB), rhinovirus (common cold), herpes simplex virus (HSV), or respiratory syncytial virus (RSV). In some embodiments, the bacterial infection is *Helicobactor pylori*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, or *Chlamydia trachomatis*. In some embodiments the parasitic, protozoan, or metazoan infection is *Schistosoma mansoni*, *Taenia crassiceps*, or *Leishmania mexicana*.

[0033] In some embodiments, the infectious disease induces PD-1 overexpression in the individual. In some embodiments, the method includes selecting the individual having PD-1 overexpression. In some embodiments, any suitable diagnostic assays are used to detect biomarkers of PD-1 overexpression. In some embodiments, an immunological assay (immunohistochemistry, IHC; antibody based cell sorting) is adapted for use with tissues, for example tumor tissue and/or immune cells (e.g., whether they are tumor infiltrating immune cells or circulating immune cells). In some embodiments, tissue is collected in a way that is applicable to histology (e.g., FFPE, fresh

frozen or smears or cell suspension) and used in immunohistochemistry stainings (e.g., usually chromogenic, but also fluorescent) or antibody based cell sorting. In some embodiments, quantitative PCR methods, as well as FISH (fluorescent in situ hybridization), and CISH (chromogenic ISH) are used for DNA mutations (e.g., such as gene recombinations, deletions, amplifications, mutations).

[0034] In some embodiments, the infectious disease is a p53 expressing infectious disease. In some embodiments, the method includes selecting the individual having p53 overexpression. In some embodiments, the method includes selecting an individual having functional p53. In some embodiments, known diagnostic assays are used to detect biomarkers. In some embodiments, an immunological assay (IHC) on tissues using a DO7 clone antibody is suitable to detect overexpressed nuclear staining of p53 which indicates mutated p53. In some embodiments, quantitative PCR methods, as well as FISH (fluorescent in situ hybridization), and CISH (chromogenic ISH) are applied.

[0035] In some embodiments, the parasite, protozoan, or metazoan causing the infectious disease is genotyped. In some embodiments, any suitable means of assaying genotype is contemplated for use with the method disclosed herein.

[0036] In some instances, T cell exhaustion occurs during chronic viral, bacterial, and parasitic infections. In some instances, blocking the PD-1:PD-L inhibitory receptor pathway reinvigorates CD8+ T cell responses. In some embodiments, the synthetic miR-34 oligonucleotide reverses, reduces, or suppresses T cell exhaustion.

Therapies

Synthetic miRNA oligonucleotides

[0037] MicroRNAs (miRNAs) are small non-coding, naturally occurring RNA molecules that post-transcriptionally modulate gene expression and determine cell fate by regulating multiple gene products and cellular pathways. miRNAs interfere with gene expression by degrading the mRNA transcript by blocking the protein translation machinery. miRNAs target mRNAs with sequences that are fully or partially complementary which endows these regulatory RNAs with the ability to target a broad but nevertheless specific set of mRNAs. To date, there are ~1,500 human annotated miRNA genes with roles in processes as diverse as cell proliferation, differentiation, apoptosis, stem cell development, and immune function. In some instances, the misregulation of miRNAs contributes to the development of human diseases, including cancer. In some instances, miRNAs deregulated in cancer function as *bona fide* tumor suppressors or oncogenes. In some instances, a single miRNA targets multiple oncogenes and oncogenic signaling pathways, and translating this

ability into a future therapeutic may hold the promise of creating a remedy that is effective against tumor heterogeneity. Thus, miRNAs have the potential of becoming powerful therapeutic agents for cancer that act in accordance with our current understanding of cancer as a "pathway disease" that is only successfully treated when intervening with multiple cancer pathways.

Synthetic miR-34 oligonucleotide

[0038] Disclosed herein, in certain embodiments, are compositions comprising: (a) a synthetic miR-34 oligonucleotide, comprising: (i) an active strand comprising a sequence at least 80% identical to a mature miRNA; and (ii) a separate passenger strand that is at least 60% complementary to the active strand; and (b) a PD-1 or PD-L1 inhibitor. In some embodiments, the passenger strand of the synthetic miR-34 oligonucleotide comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine. In some embodiments, the 5' terminal cap is NH_2-$(CH_2)_6$-O-. In some embodiments, the mature miRNA comprises: miR-34a (SEQ ID NO:1), miR-34b (SEQ ID NO:2), miR-34c (SEQ ID NO:3), or a miR-34 consensus sequence (SEQ ID NO:4). In some embodiments, the mature miRNA comprises: miR-449a (SEQ ID NO:5), miR-449b (SEQ ID NO:6), miR-449c (SEQ ID NO:7), or a miR-449 consensus sequence (SEQ ID NO:8). In some embodiments, the mature miRNA comprises a miR-34/449 seed sequence (SEQ ID NO:9). In some embodiments, the PD-1 or PD-L1 inhibitor is an antibody, small interfering RNA, or antisense RNA. In some embodiments, the synthetic miR-34 oligonucleotide and the PD-1 or PD-L1 inhibitor are synergistic. In some embodiments, the synthetic miR-34 oligonucleotide is provided before the PD-1 or PD-L1 inhibitor.

[0039] In some embodiments, the synthetic miR-34 oligonucleotide is a microRNA mimic. In some embodiments, the synthetic miR-34 oligonucleotide is administered by injection or transfusion. In some embodiments, the synthetic miR-34 oligonucleotide is provided in a vector (e.g., using a gene therapy methodology). Representative synthetic miR-34 oligonucleotide sequences are provided in Table 1 below.

Table 1: microRNA Sequences and Sequence Identification Numbers

microRNA	Sequence	SEQ ID NO:
miR-34a	U**GGCAGUG**UCUUAGCUGGUUGUU	SEQ ID NO:1
miR-34b	UA**GGCAGUG**UCAUUAGCUGAUUG	SEQ ID NO:2
miR-34c	A**GGCAGUG**UAGUUAGCUGAUUGC	SEQ ID NO:3

miR-34 consensus	*GGCAGUGU*UUAGCUG*UUG*	SEQ ID NO:4
miR-449a	UGGCAGUGUAUUGUUAGCUGGU	SEQ ID NO:5
miR-449b	AGGCAGUGUAUUGUUAGCUGGC	SEQ ID NO:6
miR-449c	UAGGCAGUGUAUUGCUAGCGGCUGU	SEQ ID NO:7
miR-449 consensus	UGGCAGUGUAUUG*UAGC*G*G	SEQ ID NO:8
miR-34/449 seed	GGCAGUG	SEQ ID NO:9

"*" denotes a deletion of any nucleotide(s). miR-34/449 seed sequences are shown in bold highlighting.

[0040] In some embodiments, the synthetic miR-34 oligonucleotide is 7-130 nucleotides long, double stranded RNA molecules. In some embodiments, a synthetic miR-34 oligonucleotide is 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 7-30, 7-25, 15-30, 15-25, 17-30, or 17-25 nucleotides long.

[0041] In some embodiments, the synthetic miR-34 oligonucleotide is two separate strands (i.e., an active strand and a separate passenger strand). In some embodiments, the synthetic miR-34 oligonucleotide is a hairpin structure.

[0042] In some embodiment, the active strand comprises or consists of a sequence which is identical or substantially identical to a mature microRNA sequence. In some embodiments, "substantially identical", as used herein, means that the sequence is at least 80% identical to the mature microRNA sequence. In some embodiments, the mature microRNA sequence is miR-34a (SEQ ID NO: 1). In some embodiments, the mature microRNA sequence is miR-34b (SEQ ID NO: 2). In some embodiments, the mature microRNA sequence is miR-34c (SEQ ID NO: 3). In some embodiments, the mature microRNA sequence is miR-449a (SEQ ID NO: 5). In some embodiments, the mature microRNA sequence is miR-449b (SEQ ID NO: 6). In some embodiments, the mature microRNA sequence is miR-449c (SEQ ID NO: 7).

[0043] In some embodiments, the active strand comprises or consists of a sequence that is at least 80% identical to miR-34a (SEQ ID NO: 1). In some embodiments, the active strand comprises or consists of a sequence that is at least 80% identical to miR-34b (SEQ ID NO: 2). In some embodiments, the active strand comprises or consists of a sequence that is at least 80% identical to miR-34c (SEQ ID NO: 3). In some embodiments, the active strand comprises or consists of a sequence that is at least 80% identical to miR-449a (SEQ ID NO: 5). In some embodiments, the active strand comprises or consists of a sequence that is at least 80% identical to miR-449b (SEQ

ID NO: 6). In some embodiments, the active strand comprises or consists of a sequence that is at least 80% identical to miR-449c (SEQ ID NO: 7). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34/449 seed sequence (SEQ ID NO: 9). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34 consensus sequence (SEQ ID NO: 4). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34 consensus sequence (SEQ ID NO: 8).

[0044] In some embodiments, the active strand comprises or consists of a sequence that is at least 85% identical to miR-34a (SEQ ID NO: 1). In some embodiments, the active strand comprises or consists of a sequence that is at least 85% identical to miR-34b (SEQ ID NO: 2). In some embodiments, the active strand comprises or consists of a sequence that is at least 85% identical to miR-34c (SEQ ID NO: 3). In some embodiments, the active strand comprises or consists of a sequence that is at least 85% identical to miR-449a (SEQ ID NO: 5). In some embodiments, the active strand comprises or consists of a sequence that is at least 85% identical to miR-449b (SEQ ID NO: 6). In some embodiments, the active strand comprises or consists of a sequence that is at least 85% identical to miR-449c (SEQ ID NO: 7). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34/449 seed sequence (SEQ ID NO: 9). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34 consensus sequence (SEQ ID NO: 4). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34 consensus sequence (SEQ ID NO: 8).

[0045] In some embodiments, the active strand comprises or consists of a sequence that is at least 90% identical to miR-34a (SEQ ID NO: 1). In some embodiments, the active strand comprises or consists of a sequence that is at least 90% identical to miR-34b (SEQ ID NO: 2). In some embodiments, the active strand comprises or consists of a sequence that is at least 90% identical to miR-34c (SEQ ID NO: 3). In some embodiments, the active strand comprises or consists of a sequence that is at least 90% identical to miR-449a (SEQ ID NO: 5). In some embodiments, the active strand comprises or consists of a sequence that is at least 90% identical to miR-449b (SEQ ID NO: 6). In some embodiments, the active strand comprises or consists of a sequence that is at least 90% identical to miR-449c (SEQ ID NO: 7). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34/449 seed sequence (SEQ ID NO: 9). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34 consensus sequence (SEQ ID

NO: 4). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34 consensus sequence (SEQ ID NO: 8).

[0046] In some embodiments, the active strand comprises or consists of a sequence that is at least 95% identical to miR-34a (SEQ ID NO: 1). In some embodiments, the active strand comprises or consists of a sequence that is at least 95% identical to miR-34b (SEQ ID NO: 2). In some embodiments, the active strand comprises or consists of a sequence that is at least 95% identical to miR-34c (SEQ ID NO: 3). In some embodiments, the active strand comprises or consists of a sequence that is at least 95% identical to miR-449a (SEQ ID NO: 5). In some embodiments, the active strand comprises or consists of a sequence that is at least 95% identical to miR-449b (SEQ ID NO: 6). In some embodiments, the active strand comprises or consists of a sequence that is at least 95% identical to miR-449c (SEQ ID NO: 7). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34/449 seed sequence (SEQ ID NO: 9). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34 consensus sequence (SEQ ID NO: 4). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34 consensus sequence (SEQ ID NO: 8).

[0047] In some embodiments, the active strand comprises or consists of a sequence that is identical to miR-34a (SEQ ID NO: 1). In some embodiments, the active strand comprises or consists of a sequence that is identical to miR-34b (SEQ ID NO: 2). In some embodiments, the active strand comprises or consists of a sequence that is identical to miR-34c (SEQ ID NO: 3). In some embodiments, the active strand comprises or consists of a sequence that is identical to miR-449a (SEQ ID NO: 5). In some embodiments, the active strand comprises or consists of a sequence that is identical to miR-449b (SEQ ID NO: 6). In some embodiments, the active strand comprises or consists of a sequence that is identical to miR-449c (SEQ ID NO: 7). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34/449 seed sequence (SEQ ID NO: 9). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34 consensus sequence (SEQ ID NO: 4). In some embodiments, the active strand comprises or consists of a sequence which is identical or substantially identical to the miR-34 consensus sequence (SEQ ID NO: 8).

[0048] In some embodiments, the passenger strand comprises a sequence that is at least 60% complementary to the active strand. In some embodiments, the passenger strand comprises a sequence that is at least 65% complementary to the active strand. In some embodiments, the passenger strand comprises a sequence that is at least 70% complementary to the active strand. In

some embodiments, the passenger strand comprises a sequence that is at least 75% complementary to the active strand. In some embodiments, the passenger strand comprises a sequence that is at least 80% complementary to the active strand. In some embodiments, the passenger strand comprises a sequence that is at least 85% complementary to the active strand. In some embodiments, the passenger strand comprises a sequence that is at least 90% complementary to the active strand. In some embodiments, the passenger strand comprises a sequence that is at least 95% complementary to the active strand. In some embodiments, the passenger strand comprises a sequence that is complementary to the active strand.

[0049] In some embodiments, the synthetic miR-34 oligonucleotide is chemically modified or designed to comprise one or more specific sequence variations. In some embodiments, synthetic miR-34 oligonucleotide has a 5' terminal cap on the passenger strand. Any suitable cap may be used with the compositions disclosed herein. In some embodiments, the synthetic miR-34 oligonucleotide comprises a lower alkylamine cap on the 5' terminus of the passenger strand. In some embodiments, the synthetic miR-34 oligonucleotide comprises aNH_2-$(CH_2)_6$-O- cap on the 5' terminus of the passenger strand. In some embodiments, the synthetic miR-34 oligonucleotide comprises a mismatch at the first and/or second nucleotide of the passenger strand. In some embodiments, at least one nucleotide of the passenger strand comprises a sugar modification. In some embodiments, at least one nucleotide of the active strand comprises a sugar modification. In some embodiments, at least one nucleotide of the passenger strand and at least one nucleotide if the active strand comprises a sugar modification. Additional non-limiting examples of chemical modifications include backbone modifications (e.g., phosphorothioate, morpholinos), ribose modifications (e.g., 2'-OMe, 2'-Me, 2'-F, 2'-4'-locked/bridged sugars (e.g., LNA, ENA, UNA), and nucleobase modifications.

[0050] In some embodiments, the synthetic miR-34 oligonucleotide is between 17 and 30 nucleotides in length and comprises (i) an active strand comprising or consisting of a sequence from 5' to 3' that is at least 80% identical to SEQ ID NO: 1, and (ii) a separate passenger strand comprising a sequence from 5' to 3' that is at least 60% complementary to the active strand. In some embodiments, the passenger strand comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine.

[0051] In some embodiments, the synthetic miR-34 oligonucleotide is between 17 and 30 nucleotides in length and comprises (i) an active strand comprising or consisting of a sequence from 5' to 3' that is at least 80% identical to SEQ ID NO: 2, and (ii) a separate passenger strand comprising or consisting of a sequence from 5' to 3' that is at least 60% complementary to the

active strand. In some embodiments, the passenger strand comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine.

[0052] In some embodiments, the synthetic miR-34 oligonucleotide is between 17 and 30 nucleotides in length and comprises (i) an active strand comprising or consisting of a sequence from 5' to 3' that is at least 80% identical to SEQ ID NO: 3, and (ii) a separate passenger strand comprising or consisting of a sequence from 5' to 3' that is at least 60% complementary to the active strand. In some embodiments, the passenger strand comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine.

[0053] In some embodiments, the synthetic miR-34 oligonucleotide is between 17 and 30 nucleotides in length and comprises (i) an active strand comprising or consisting of a sequence from 5' to 3' that is at least 80% identical to SEQ ID NO: 4, and (ii) a separate passenger strand comprising or consisting of a sequence from 5' to 3' that is at least 60% complementary to the active strand. In some embodiments, the passenger strand comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine.

[0054] In some embodiments, the synthetic miR-34 oligonucleotide is between 17 and 30 nucleotides in length and comprises (i) an active strand comprising or consisting of a sequence from 5' to 3' that is at least 80% identical to SEQ ID NO: 5, and (ii) a separate passenger strand comprising or consisting of a sequence from 5' to 3' that is at least 60% complementary to the active strand. In some embodiments, the passenger strand comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine.

[0055] In some embodiments, the synthetic miR-34 oligonucleotide is between 17 and 30 nucleotides in length and comprises (i) an active strand comprising or consisting of a sequence from 5' to 3' that is at least 80% identical to SEQ ID NO: 6, and (ii) a separate passenger strand comprising or consisting of a sequence from 5' to 3' that is at least 60% complementary to the active strand. In some embodiments, the passenger strand comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine.

[0056] In some embodiments, the synthetic miR-34 oligonucleotide is between 17 and 30 nucleotides in length and comprises (i) an active strand comprising or consisting of a sequence from 5' to 3' that is at least 80% identical to SEQ ID NO: 7, and (ii) a separate passenger strand comprising or consisting of a sequence from 5' to 3' that is at least 60% complementary to the active strand. In some embodiments, the passenger strand comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine.

[0057] In some embodiments, the synthetic miR-34 oligonucleotide is between 17 and 30 nucleotides in length and comprises (i) an active strand comprising or consisting of a sequence

from 5' to 3' that is at least 80% identical to SEQ ID NO: 8, and (ii) a separate passenger strand comprising or consisting of a sequence from 5' to 3' that is at least 60% complementary to the active strand. In some embodiments, the passenger strand comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine.

[0058] In some embodiments, the synthetic miR-34 oligonucleotide is between 17 and 30 nucleotides in length and comprises (i) an active strand comprising or consisting of a sequence from 5' to 3' that is at least 80% identical to SEQ ID NO: 9, and (ii) a separate passenger strand comprising or consisting of a sequence from 5' to 3' that is at least 60% complementary to the active strand. In some embodiments, the passenger strand comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine.

[0059] In some embodiments, the synthetic miR-34 oligonucleotide comprises a sequence that is at least 80% identical to at least one of SEQ ID NO:1-9. In some embodiments, the synthetic miR-34 oligonucleotide comprises a sequence that is at least 85% identical to at least one of SEQ ID NO:1-9. In some embodiments, the synthetic miR-34 oligonucleotide comprises a sequence that is at least 90% identical to at least one of SEQ ID NO:1-9. In some embodiments, the synthetic miR-34 oligonucleotide comprises a sequence that is at least 95% identical to at least one of SEQ ID NO:1-9. In some embodiments, the synthetic miR-34 oligonucleotide comprises a sequence that is at least 100% identical to at least one of SEQ ID NO:1-9. In some embodiments, the synthetic miR-34 oligonucleotide comprises a sequence that differs from at least one of SEQ ID NO:1-9 by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleobases.

[0060] In some embodiments, the synthetic miR-34 oligonucleotide comprises a single polynucleotide or a double stranded polynucleotide. In some embodiments, the synthetic miR-34 oligonucleotide comprises a hairpin polynucleotide.

[0061] In some embodiments, the synthetic miR-34 oligonucleotide is between 17 and 30 nucleotides in length and comprises one or more of the following (i) a 5' terminal cap on the passenger strand; (ii) one or more sugar modifications in the first or last 1 to 6 residues of the passenger strand; or (iii) non-complementarity between one or more nucleotides in the last 1 to 5 residues at the 3' end of the passenger strand and the corresponding nucleotides of the active strand.

[0062] In some embodiments, the synthetic miR-34 oligonucleotide is between 17 and 30 nucleotides in length and comprises (i) at least one modified nucleotide that blocks the 5' OH or phosphate at the 5' terminus of the passenger strand, wherein the at least one nucleotide modification is an NH_2, biotin, an amine group, a lower alkylamine group, an acetyl group or 2'oxygen-methyl (2'O-Me) modification; or (ii) at least one ribose modification to the active strand or the passenger strand selected from 2'F, 2'NH_2, 2'N_3, 4'thio, or 2'O-CH_3.

[0063] In some embodiments, the synthetic miR-34 oligonucleotide further comprises a complementary strand that is at least 60% complementary to the synthetic miR-34 oligonucleotide. In some embodiments, the complementary strand is not naturally occurring. In some embodiments, the complementary strand comprises (a) a chemical modification that improves uptake of the synthetic miR-34 oligonucleotide, (b) a chemical modification that enhances activity of the synthetic miR-34 oligonucleotide, (c) a chemical modification that enhances stability of the synthetic miR-34 oligonucleotide, (d) a chemical modification that inhibits uptake of the complementary strand, (e) a chemical modification that inhibits activity of the complementary strand. In some embodiments, the complementary strand comprises one or more nucleobases that are non-complementary with the synthetic miR-34 oligonucleotide.

PD-1 or PD-L1 inhibitors

[0064] Programmed cell death protein 1 (PDCD1, PD1, PD-1) is a cell surface receptor that belongs to the immunoglobulin superfamily and is expressed on T cells and pro-B cells. In humans, PD-1 is encoded by the PDCD1 gene. PD-1 binds two ligands, PD-L1 and PD-L2. PD-1 and its ligands play an important role in down regulating the immune system by preventing the activation of T-cells, which in turn can reduce autoimmunity and promote self-tolerance. In some instances, the inhibitory effect of PD-1 is accomplished through a dual mechanism of promoting apoptosis (programmed cell death) in antigen specific T-cells in lymph nodes while simultaneously reducing apoptosis in regulatory T cells (suppressor T cells).

[0065] PD-1 is a type I membrane protein of 268 amino acids. PD-1 is a member of the extended CD28/CTLA-4 family of T cell regulators. The protein's structure includes an extracellular IgV domain followed by a transmembrane region and an intracellular tail. The intracellular tail contains two phosphorylation sites located in an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif, which suggests that PD-1 negatively regulates TCR signals. This is consistent with binding of SHP-1 and SHP-2 phosphatases to the cytoplasmic tail of PD-1 upon ligand binding. PD-1 is expressed on the surface of activated T cells, B cells, and macrophages, suggesting that compared to CTLA-4, PD-1 more broadly negatively regulates immune responses.

[0066] Programmed death-ligand 1 (PDL1, PD-L1, and also known as CD274 – cluster of differentiation 274 or B7-H1 – B7 homolog 1) is encoded by the CD274 gene in humans. PD-L1 is a 40 kDa type 1 transmembrane protein that has been speculated to play a major role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states such as hepatitis. Normally, the immune system reacts to foreign antigens where there is some accumulation in the lymph nodes or spleen which triggers a

proliferation of antigen-specific CD8+ T cell. The formation of PD-1 receptor / PD-L1 or B7.1 receptor /PD-L1 ligand complex transmits an inhibitory signal which reduces the proliferation of these CD8+ T cells at the lymph nodes and supplementary to that PD-1 is also able to control the accumulation of foreign antigen specific T cells in the lymph nodes through apoptosis which is further mediated by a lower regulation of the gene Bcl-2.

[0067] In some embodiments, the methods use a PD-1 or PD-L1 inhibitor. In some embodiments, PD-1 or PD-L1 inhibitors include molecules that specifically interact with PD-1 or PD-L1 to inhibit immune system downregulation. In some embodiments, PD-1 or PD-L1 inhibitors are antibodies (e.g., commercially available antibodies, or other antibodies produced against PD-1 or PD-L1 – see Table 2 below for non-limiting examples), small molecule drugs, small interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), antisense RNAs (such as locked nucleic acids, LNA), or interfering DNA molecules (DNAi).

Table 2: Selected PD-1 and PD-L1 inhibitors.

Drug	Developer	Target
Opdivo (nivolumab; fully human IgG4 antibody)	BMS, Ono	PD-1
Keytruda (pembrolizumab; humanized IgG4 antibody)	Merck	PD-1
Atezolizumab (MPDL3280A; RG7446; IgG1k antibody)	Roche	PD-L1
Durvalumab (MEDI4736; IgG1k antibody)	AstraZeneca	PD-L1
Pidilizumab (CT-011)	Curetech (Yavne, Israel)	PD-1
Avelumab (MSB0010718C; fully human IgG1 antibody)	Merck Serono	PD-L1
AMP-224 (an Fc–PD-L2 fusion protein that blocks PD-1 from binding its partners)	GlaxoSmithKline	PD-1
BMS-936559 (MDX-1105; fully human IgG4 antibody)	BMS	PD-L1

Radiotherapy

[0068] Radiotherapy (e.g., radiation therapy, abbreviated RT, RTx, or XRT) is a therapy using ionizing radiation to control or kill malignant cells by damaging the DNA of cancerous tissue. In some embodiments, radiotherapy is administered with the intent to cure, as an adjuvant, as a neoadjuvant, for therapeutic purposes, or for palliative purposes. The purpose for which

radiotherapy is administered is dependent on the tumor type, tumor location, tumor stage, and health of the individual. In some embodiments, radiation therapy is synergistic with another therapy (e.g., immunotherapy, chemotherapy, microRNA therapy), and is used before, during, and after the other therapy.

[0069] Ionizing radiation includes photon radiation (e.g. x-rays and gamma rays) or particle radiation (e.g. electrons, protons, neutrons, carbon ions, alpha particles, and beta particles). In photon radiation, radiation comes from a radioactive source such as cobalt, cesium, or a linear accelerator.

[0070] In some embodiments, radiotherapy is delivered using external beam radiation therapy, brachytherapy, or systemic radioisotope therapy. In some embodiments, external beam radiation therapy is three-dimensional conformal radiation therapy (3D-CRT), intensity-modulated radiation therapy (IMRT), image-guided radiation therapy (IGRT), intensity modulated proton therapy (IMPT), stereotactic radiosurgery (SRS), stereotactic body radiation therapy (SBRT), intraoperative radiation therapy (IORT), or electromagnetic-guided radiation therapy. In some embodiments, brachytherapy radiation is interstitial radiation or intracavitary radiation. In some embodiments, brachytherapy is permanent or temporary. In some embodiments, temporary brachytherapy is high-dose rate (HDR) or low-dose rate (LDR)

[0071] An absorbed dose of radiation is measured in gray (Gy). In curative cases, the typical dose for a solid epithelial tumor ranges from 40 to 80 Gy. Preventive (adjuvant) doses are typically around 45–60 Gy in 1.8–2 Gy fractions (e.g., for breast, head, and neck cancers.) However, many other factors are considered by radiation oncologists when selecting a dose, including whether the patient is receiving a combination therapy, patient comorbidities, whether radiation therapy is being administered before or after surgery or combination therapy, and the degree of success of the surgery or combination therapy.

[0072] Delivery parameters of a prescribed dose are determined during treatment planning (part of dosimetry). In some embodiments, treatment planning is performed on dedicated computers using specialized treatment planning software. In some embodiments, several angles or sources are used to sum to the total necessary dose. In some embodiments, a planner designs a treatment plan for an individual in need thereof. In some embodiments, the planner is a physician, radiation oncologist, or other health care professional. In some embodiments, the treatment plans calls for a uniform prescription dose of radiotherapy to the tumor. In some embodiments, for clinically approved radiotherapy, a treatment plan is adapted from the approved clinical protocol. In some embodiments, for new radiotherapy methods, a treatment plan is developed using established

clinical trial protocols. In some embodiments, radiotherapy treatments are designed and implemented, for example, by radiation oncologists.

Administration of therapies

Monotherapy

[0073] Disclosed herein, in certain embodiments, are methods of treating a PD-L1 expressing cancer in an individual in need thereof comprising: (a) identifying the cancer as a PD-L1 expressing cancer; and (b) providing a synthetic miR-34 oligonucleotide to the individual. In some embodiments, the methods further comprise providing a PD-1 or PD-L1 inhibitor to the individual. In some embodiments, the PD-1 or PD-L1 inhibitor is an antibody, small interfering RNA, or antisense RNA. In some embodiments, the synthetic miR-34 oligonucleotide and the PD-1 or PD-L1 inhibitor are synergistic. In some embodiments, the synthetic miR-34 oligonucleotide is provided before the PD-1 or PD-L1 inhibitor. In some embodiments, the methods further comprise providing radiotherapy to the individual. In some embodiments, the synthetic miR-34 oligonucleotide is provided before the radiotherapy. In some embodiments, the cancer is a solid tumor. In some embodiments, the solid cancer is a melanoma, nasopharyngeal cancer, neuroendrocrine tumor, lung cancer, colon cancer, urothelial cancer, bladder cancer, liver cancer, multiple myeloma, ovarian cancer, gastric carcinoma, esophageal cancer, pancreatic cancer, kidney cancer, breast cancer, or lymphoma. In some embodiments, the lung cancer is a non-small cell lung cancer (NSCLC). In some embodiments, the lung cancer is a small-cell lung cancer (SCLC). In some embodiments, the liver cancer is a hepatocellular carcinoma (HCC). In some embodiments, the cancer is a leukemia. In some embodiments, the cancer is a lymphoma. In some embodiments, the cancer as a PD-L1 expressing cancer comprises measuring the PD-L1 expression in a cancer cell from the individual and comparing to a control. In some embodiments, the PD-L1 expression is overexpressed compared to the control. In some embodiments, the methods further comprise selecting the individual having functional p53. In some embodiments, the synthetic miR-34 oligonucleotide is administered in a liposomal formulation. In some embodiments, T cell exhaustion is prevented or reduced. In some embodiments, the synthetic miR-34 oligonucleotide comprises: (a) an active strand comprising a sequence at least 80% identical to a mature miRNA; and (b) a separate passenger strand that is at least 60% complementary to the active strand. In some embodiments, the passenger strand of the synthetic miR-34 oligonucleotide comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine. In some embodiments, the 5' terminal cap is NH_2-$(CH_2)_6$-O-. In some embodiments, the mature miRNA comprises: miR-34a (SEQ ID NO:1), miR-34b (SEQ ID NO:2), miR-34c (SEQ ID NO:3), or a miR-34 consensus

sequence (SEQ ID NO:4). In some embodiments, the mature miRNA comprises: miR-449a (SEQ ID NO:5), miR-449b (SEQ ID NO:6), miR-449c (SEQ ID NO:7), or a miR-449 consensus sequence (SEQ ID NO:8). In some embodiments, the mature miRNA comprises a miR-34/449 seed sequence (SEQ ID NO:9).

[0074] In some embodiments, a dosage amount is an amount of the synthetic miR-34 oligonucleotide administered to an individual in need thereof. In some embodiments, a dosing schedule is the schedule during which the synthetic miR-34 oligonucleotide is administered to an individual in need thereof.

[0075] In some embodiments, the synthetic miR-34 oligonucleotide is continued until a clinical endpoint is met. In some embodiments, the synthetic miR-34 oligonucleotide is continued until disease progression or unacceptable toxicity occurs.

[0076] In some embodiments, the synthetic miR-34 oligonucleotide is continued until achieving a pathological complete response (pCR) rate defined as the absence of the cancer being treated. In some embodiments, the synthetic miR-34 oligonucleotide is continued until partial or complete remission of the cancer. In some embodiments, the synthetic miR-34 oligonucleotide is continued until partial or complete remission of the liver cancer. In some embodiments, the synthetic miR-34 oligonucleotide reduces the size or number of the cancer tumor(s). In some embodiments, the synthetic miR-34 oligonucleotide prevents the cancer tumor(s) from increasing in size and/or number. In some embodiments, the synthetic miR-34 oligonucleotide prevents the cancer tumor(s) from metastasizing.

[0077] In some embodiments, the synthetic miR-34 oligonucleotide is continued until the viral, bacterial, or parasitic/protozoan/metazoan infection is eliminated.

[0078] In some embodiments, administration of the synthetic miR-34 oligonucleotide is not limited to any particular delivery system and includes, without limitation, parenteral (including subcutaneous, intravenous, intramedullary, intraarticular, intramuscular, or intraperitoneal injection), rectal, topical, transdermal, or oral (for example, in capsules, suspensions, or tablets) administration. In some embodiments, administration to an individual in need thereof occurs in a single dose or in repeat administrations, and in any of a variety of physiologically acceptable salt forms, or with an acceptable pharmaceutical carrier or additive as part of a pharmaceutical composition. In some embodiments, any suitable and physiological acceptable salt forms or standard pharmaceutical formulation techniques, dosages, and excipients are utilized.

[0079] In some embodiments, effective dosages achieved in one animal are extrapolated for use in another animal, including humans, using conversion factors known in the art. See Table 3 for equivalent surface area dosage factors.

Table 3: equivalent surface area dosage factors

From: To:	Mouse (20 g)	Rat (150 g)	Monkey (3.5 kg)	Dog (8 kg)	Human (60 kg)
Mouse	1	0.5	0.25	0.17	0.08
Rat	2	1	0.5	0.25	0.14
Monkey	4	2	1	0.6	0.33
Dog	6	4	1.7	1	0.5
Human	12	7	3	2	1

[0080] In some embodiments, the synthetic miR-34 oligonucleotide dosing amount or schedule follows clinically approved, or experimental, guidelines. In some embodiments, the dose of oligonucleotide is about 10, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, or 250 mg/m^2 per day.

[0081] In some embodiments the synthetic miR-34 oligonucleotide is administered to the individual in 1, 2, 3, 4, 5 daily doses over 5 days. In some embodiments, the synthetic miR-34 oligonucleotide is administered to the individual in 1, 2, 3, 4, 5, 6, or 7 daily doses over a single week (7 days). In some embodiments, the synthetic miR-34 oligonucleotide is administered to the individual in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 daily doses over 14 days. In some embodiments, the synthetic miR-34 oligonucleotide is administered to the individual in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 daily doses over 21 days. In some embodiments, the synthetic miR-34 oligonucleotide is administered to the individual in 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, or 28 daily doses over 28 days.

[0082] In some embodiments, the synthetic miR-34 oligonucleotide is provided twice a week of a 21 or a 28 day cycle. In particular embodiments, the synthetic miR-34 oligonucleotide is provided on days 1, 4, 8, 11, 15 and 18 of a 21 day or 28 day cycle.

[0083] In some embodiments the synthetic miR-34 oligonucleotide is administered for: 2 weeks (total 14 days); 1 week with 1 week off (total 14 days); 3 consecutive weeks (total 21 days); 2 weeks with 1 week off (total 21 days); 1 week with 2 weeks off (total 21 days); 4 consecutive weeks (total 28 days); 3 consecutive weeks with 1 week off (total 28 days); 2 weeks with 2 weeks off (total 28 days); 1 week with 3 consecutive weeks off (total 28 days).

[0084] In some embodiments the synthetic miR-34 oligonucleotide is: administered on day 1 of a 7, 14, 21 or 28 day cycle; administered on days 1 and 15 of a 21 or 28 day cycle; administered on

days 1, 8, and 15 of a 21 or 28 day cycle; or administered on days 1, 2, 8, and 15 of a 21 or 28 day cycle. In some embodiments, the synthetic miR-34 oligonucleotide is administered once every 1, 2, 3, 4, 5, 6, 7, or 8 weeks. In some embodiments, the synthetic miR-34 oligonucleotide is administered for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 cycles.

Combination therapy

[0085] Disclosed herein, in certain embodiments, are methods of treating a PD-L1 expressing cancer in an individual in need thereof comprising: (a) providing a synthetic miR-34 oligonucleotide to the individual; and (b) providing a PD-1 or PD-L1 inhibitor to the individual. In some embodiments, the PD-1 or PD-L1 inhibitor is an antibody, small interfering RNA, or antisense RNA. In some embodiments, the synthetic miR-34 oligonucleotide and the PD-1 or PD-L1 inhibitor are synergistic. In some embodiments, the synthetic miR-34 oligonucleotide is provided before the PD-1 or PD-L1 inhibitor. In some embodiments, the methods further comprise providing radiotherapy to the individual. In some embodiments, the synthetic miR-34 oligonucleotide is provided before the radiotherapy. In some embodiments, the cancer is a solid tumor. In some embodiments, the solid cancer is a melanoma, nasopharyngeal cancer, neuroendrocrine tumor, lung cancer, colon cancer, urothelial cancer, bladder cancer, liver cancer, multiple myeloma, ovarian cancer, gastric carcinoma, esophageal cancer, pancreatic cancer, kidney cancer, breast cancer, or lymphoma. In some embodiments, the lung cancer is a non-small cell lung cancer (NSCLC). In some embodiments, the lung cancer is a small-cell lung cancer (SCLC). In some embodiments, the liver cancer is a hepatocellular carcinoma (HCC). In some embodiments, the cancer is a leukemia. In some embodiments, the cancer is a lymphoma. In some embodiments, identifying the cancer as a PD-L1 expressing cancer comprises measuring the PD-L1 expression in a cancer cell from the individual and comparing to a control. In some embodiments, the PD-L1 expression is overexpressed compared to the control. In some embodiments, the methods further comprise selecting the individual having functional p53. In some embodiments, the synthetic miR-34 oligonucleotide is administered in a liposomal formulation. In some embodiments, T cell exhaustion is prevented or reduced. In some embodiments, the synthetic miR-34 oligonucleotide comprises: (a) an active strand comprising a sequence at least 80% identical to a mature miRNA; and (b) a separate passenger strand that is at least 60% complementary to the active strand. In some embodiments, the passenger strand of the synthetic miR-34 oligonucleotide comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine. In some embodiments, the 5' terminal cap is NH_2-$(CH_2)_6$-O-. In some embodiments, the mature miRNA comprises: miR-34a (SEQ ID NO:1), miR-34b (SEQ ID NO:2), miR-34c (SEQ ID NO:3), or a miR-34 consensus sequence (SEQ ID NO:4). In some embodiments, the mature miRNA comprises: miR-449a (SEQ

ID NO:5), miR-449b (SEQ ID NO:6), miR-449c (SEQ ID NO:7), or a miR-449 consensus sequence (SEQ ID NO:8). In some embodiments, the mature miRNA comprises a miR-34/449 seed sequence (SEQ ID NO:9).

[0086] Disclosed herein, in certain embodiments, are methods of treating an individual suffering from a chronic infectious disease comprising: (a) providing a synthetic miR-34 oligonucleotide to the individual; and (b) providing a PD-1 or PD-L1 inhibitor to the individual. In some embodiments, the PD-1 or PD-L1 inhibitor is an antibody, small interfering RNA, or antisense RNA. In some embodiments, the synthetic miR-34 oligonucleotide and the PD-1 or PD-L1 inhibitor are synergistic. In some embodiments, the synthetic miR-34 oligonucleotide is provided before the PD-1 or PD-L1 inhibitor. In some embodiments, the infectious disease is a persistent viral infection. In some embodiments, the viral infection is human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), rhinovirus (common cold), herpes simplex virus (HSV), or respiratory syncytial virus (RSV). In some embodiments, the infectious disease is a persistent bacterial infection. In some embodiments, the bacterial infection is *Helicobacter pylori*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, or *Chlamydia trachomatis*. In some embodiments, the infectious disease is the result of infection by a parasite, protozoan, or metazoan. In some embodiments, the parasite, protozoan, or metazoan is *Schistosoma mansoni*, *Taenia crassiceps*, or *Leishmania mexicana*. In some embodiments, the methods further comprise selecting the individual having functional p53. In some embodiments, the synthetic miR-34 oligonucleotide is administered in a liposomal formulation. In some embodiments, T cell exhaustion is prevented or reduced. In some embodiments, the synthetic miR-34 oligonucleotide comprises: (a) an active strand comprising a sequence at least 80% identical to a mature miRNA; and (b) a separate passenger strand that is at least 60% complementary to the active strand. In some embodiments, the passenger strand of the synthetic miR-34 oligonucleotide comprises a 5' terminal cap. In some embodiments, the 5' terminal cap is a lower alkylamine. In some embodiments, the 5' terminal cap is NH_2-$(CH_2)_6$-O-. In some embodiments, the mature miRNA comprises: miR-34a (SEQ ID NO:1), miR-34b (SEQ ID NO:2), miR-34c (SEQ ID NO:3), or a miR-34 consensus sequence (SEQ ID NO:4). In some embodiments, the mature miRNA comprises: miR-449a (SEQ ID NO:5), miR-449b (SEQ ID NO:6), miR-449c (SEQ ID NO:7), or a miR-449 consensus sequence (SEQ ID NO:8). In some embodiments, the mature miRNA comprises a miR-34/449 seed sequence (SEQ ID NO:9).

[0087] In some embodiments, the combination therapy is not limited to a particular course or regimen and are employed separately or in conjunction with other therapeutic modalities (e.g., chemotherapy or radiotherapy). In some embodiments, a combination therapy includes additional

therapies (e.g., pharmaceutical, radiation, and the like) beyond a PD-1 or PD-L1 inhibitor and oligonucleotide. In some embodiments, the compositions and combinations described herein are used as an adjuvant therapy (e.g., when combined with surgery). In some embodiments, the individual is also treated by surgical resection, percutaneous ethanol or acetic acid injection, transcatheter arterial chemoembolization, radiofrequency ablation, laser ablation, cryoablation, focused external beam radiation stereotactic radiotherapy, selective internal radiation therapy, intra-arterial iodine-131–lipiodol administration, and/or high intensity focused ultrasound.

[0088] Any therapy administered to the individual in addition to the synthetic miR-34 oligonucleotide is an additional therapeutic agent. In some embodiments, the additional therapeutic agent is a PD-1 or PD-L1 inhibitor, radiation, chemotherapy, an additional pharmaceutical agent, or a combination thereof.

[0089] In some embodiments, a dosage amount is an amount of the synthetic miR-34 oligonucleotide or additional therapeutic agent administered to an individual in need thereof. In some embodiments, a dosing schedule is the schedule during which the synthetic miR-34 oligonucleotide or additional therapeutic agent is administered to an individual in need thereof.

[0090] In some embodiments, the combination therapy is continued until a clinical endpoint is met. In some embodiments, the combination therapy is continued until disease progression or unacceptable toxicity occurs.

[0091] In some embodiments, the combination therapy is continued until achieving a pathological complete response (pCR) rate defined as the absence of the cancer being treated. In some embodiments, the combination therapy is continued until partial or complete remission of the cancer. In some embodiments, the combination therapy is continued until partial or complete remission of the liver cancer. In some embodiments, the combination therapy reduces the size or number of the cancer tumor(s). In some embodiments, the combination therapy prevents the cancer tumor(s) from increasing in size or number. In some embodiments, the combination therapy prevents the cancer tumor(s) from metastasizing.

[0092] In some embodiments, the combination therapy is continued until the viral, bacterial, or parasitic/protozoan/metazoan infection is eliminated.

[0093] In some embodiments, administration of the combination therapy is not limited to any particular delivery system and includes, without limitation, parenteral (including subcutaneous, intravenous, intramedullary, intraarticular, intramuscular, or intraperitoneal injection), rectal, topical, transdermal, or oral (for example, in capsules, suspensions, or tablets) administration. In some embodiments, administration to an individual in need thereof occurs in a single dose or in repeat administrations, and in any of a variety of physiologically acceptable salt forms, or with an

acceptable pharmaceutical carrier or additive as part of a pharmaceutical composition. In some embodiments, any suitable and physiological acceptable salt forms or standard pharmaceutical formulation techniques, dosages, and excipients are utilized.

[0094] In some embodiments, effective dosages achieved in one animal are extrapolated for use in another animal, including humans, using conversion factors known in the art. See Table 3 for equivalent surface area dosage factors.

[0095] In some embodiments, the synthetic miR-34 oligonucleotide dosing amount or schedule follows clinically approved, or experimental, guidelines. In some embodiments, the dose of oligonucleotide is about 10, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, or 250 mg/m^2 per day.

[0096] In some embodiments the synthetic miR-34 oligonucleotide is administered to the individual in 1, 2, 3, 4, 5 daily doses over 5 days. In some embodiments, the oligonucleotide is administered to the individual in 1, 2, 3, 4, 5, 6, or 7 daily doses over a single week (7 days). In some embodiments, the synthetic miR-34 oligonucleotide is administered to the individual in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 daily doses over 14 days. In some embodiments, the synthetic miR-34 oligonucleotide is administered to the individual in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 daily doses over 21 days. In some embodiments, the synthetic miR-34 oligonucleotide is administered to the individual in 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, or 28 daily doses over 28 days.

[0097] In some embodiments, the synthetic miR-34 oligonucleotide is provided twice a week of a 21 or a 28 day cycle. In particular embodiments, the synthetic miR-34 oligonucleotide is provided on days 1, 4, 8, 11, 15 and 18 of a 21 day or 28 day cycle.

[0098] In some embodiments the synthetic miR-34 oligonucleotide is administered for: 2 weeks (total 14 days); 1 week with 1 week off (total 14 days); 3 consecutive weeks (total 21 days); 2 weeks with 1 week off (total 21 days); 1 week with 2 weeks off (total 21 days); 4 consecutive weeks (total 28 days); 3 consecutive weeks with 1 week off (total 28 days); 2 weeks with 2 weeks off (total 28 days); 1 week with 3 consecutive weeks off (total 28 days).

[0099] In some embodiments the synthetic miR-34 oligonucleotide is: administered on day 1 of a 7, 14, 21 or 28 day cycle; administered on days 1 and 15 of a 21 or 28 day cycle; administered on days 1, 8, and 15 of a 21 or 28 day cycle; or administered on days 1, 2, 8, and 15 of a 21 or 28 day cycle. In some embodiments, the synthetic miR-34 oligonucleotide is administered once every 1, 2, 3, 4, 5, 6, 7, or 8 weeks. In some embodiments, the synthetic miR-34 oligonucleotide (and hence the combination therapy) is administered for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 cycles.

[0100] In some embodiments, a dose of radiotherapy is 40 to 80 Gy. In some embodiments, the dose of radiotherapy is divided into fractions. In some embodiments the fraction is 1.8 to 2 Gy. In some embodiments, a fraction of radiotherapy is administered to the individual once a day. In some embodiments, a fraction of radiotherapy is administered to the individual multiple times a day. In some embodiments, radiotherapy is administered to the individual for five, six, seven, or eight weeks. In some embodiments, the dose of radiotherapy is 45 to 60 Gy in 1.8 to 2 Gy fractions. In some embodiments, the dose of radiotherapy is determined by a physician or radiation oncologist. In some embodiments, the radiotherapy is administered before, during, or after surgery. In some embodiments, the radiotherapy is administered before or after combination therapy.

[0101] In some embodiments, a dosage amount of the additional therapeutic agent has a specific ratio of the components of the combination therapy (e.g., PD-1 or PD-L1 inhibitor: synthetic miR-34 oligonucleotide). In some embodiments, the dose of the synthetic miR-34 oligonucleotide is set, within a therapeutically effective range, based upon a selected ratio and dose of the additional therapeutic agent (e.g. PD-1 or PD-L1 inhibitor).

[0102] In some embodiments, the ratio of the components of the combination therapy (e.g., PD-1 or PD-L1 inhibitor:miR-34 oligonucleotide) are determined over different periods of time. In some embodiments, the ratio of PD-1 or PD-L1 inhibitor:synthetic miR-34 oligonucleotide is determined for a single day, a single week, 14 days, 21 days, or 28 days.

[0103] In some embodiments, dosing or administration of a PD-1 or PD-L1 inhibitor will vary depending upon the specific PD-1 or PD-L1 inhibitor used. In some embodiments, for clinically approved PD-1 or PD-L1 inhibitors, dosing or administration is adapted from the approved clinical protocol or experimental guidelines. In some embodiments, for new PD-1 or PD-L1 inhibitors, dosing or administration is developed using established clinical trial protocols or experimental guidelines.

[0104] In some embodiments, the PD-1 or PD-L1 inhibitor schedule follows clinically approved or experimental guidelines.

[0105] In some embodiments, the synthetic miR-34 oligonucleotide is administered prior to the PD-1 or PD-L1 inhibitor, concurrently with the PD-1 or PD-L1 inhibitor, after the PD-1 or PD-L1 inhibitor, or a combination thereof. In some embodiments, the synthetic miR-34 oligonucleotide is administered intravenously. In some embodiments, the synthetic miR-34 oligonucleotide is administered systemically or regionally.

[0106] In some embodiments, the combination of the synthetic miR-34 oligonucleotide and PD-1 or PD-L1 inhibitor is used as an adjuvant, neoadjuvant, concomitant, concurrent, or palliative

therapy. In some embodiments, the combination of the synthetic miR-34 oligonucleotide and PD-1 or PD-L1 inhibitor is used as a first line therapy, second line therapy, or crossover therapy.

[0107] In some embodiments, the therapeutically effective dose of PD-1 or PD-L1 inhibitor is reduced through combination with the synthetic miR-34 oligonucleotide. In some embodiments, the weekly or monthly dose of PD-1 or PD-L1 inhibitor is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more relative to the maximum recommended dose or the maximum tolerated dose. In other embodiments, the PD-1 or PD-L1 inhibitor is administered at an effective dose that at least 50%, 60%, 70%, 80%, 90% or more below the dose needed to be effective in the absence of the synthetic miR-34 oligonucleotide administration. In some embodiments, the IC50 of the PD-1 or PD-L1 inhibitor is reduced by at least 4-, 5-, 10-, 20-, 30-, 40-, 50-, or 100-fold relative to the IC50 in the absence of the synthetic miR-34 oligonucleotide.

Synergy and combination index (CI) values

[0108] While synergy and synonymous terms are commonly used in the art, the property is not always defined or quantified (and, hence, the purported synergy may not actually be present). In some embodiments, combination index (CI) values are used to quantify the effects of various combination therapies (e.g., combinations of PD-1 or PD-L1 inhibitor and oligonucleotides). In some embodiments, the combination therapy exhibits synergy, for example, as quantified by a combination index (CI) < 1. In some embodiments, the combination index (CI) is less than about 0.80, 0.75, 0.70, 0.65, 0.60, 0.55, 0.50, 0.45, 0.40, 0.35, 0.30, 0.25, or 0.20.

[0109] In some embodiments, CI values are based on Loewe's additivity model to determine the nature of drug-drug interactions as additive (CI=1), antagonistic (CI>1), or synergistic (CI<1) for various drug-drug concentrations and effect levels (Fa, fraction affected; inhibition of cancer cell proliferation). In some embodiments, CI values are calculated based on linear regression trendlines using the CompuSyn software (ComboSyn Inc., Paramus, NJ), whereby the hyperbolic and sigmoidal dose-effect curves are transformed into a linear form.

[0110] In some embodiments, the combination therapy (e.g., the ratio of PD-1 or PD-L1 inhibitor:oligonucleotide) exhibits a CI<1. In some embodiments, the combination therapy has a CI < 0.95, 0.90, 0.85, 0.80, 0.75, 0.70, 0.65, 0.60, 0.55, 0.50, 0.45, 0.40, 0.35, 0.30, 0.25, or 0.20. In some embodiments, CI is used in conjunction with other parameters, for example CI < 0.60, Dose Reduction Index (DRI) > 2, and fraction affected by the dose (Fa) > 65% to define a synergistic therapy. In some embodiments, the CI < 0.80, 0.75, 0.70, 0.65, 0.60, 0.55, or 0.50 (and optionally in combination with other parameters, for example DRI > 2, and Fa > 65%).

[0111] CI values cannot, practically speaking, be calculated in a human because that would require providing the therapy in serial dilution (e.g., generally be considered unethical). Therefore, in some

embodiments, for human therapy, the CI value is considered to be the CI value of a reference system – a non-limiting example being a cell assay or an animal model.

EXAMPLES

Example 1: Investigation of relationship between p53 and miR-34a with PD-L1 expression

Methods

Cell Lines

[0112] Lung cancer cell lines A549, H460, H1299 were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and validated by MD Anderson's Characterized Cell Line Core facility by short-tandem-repeat (STR) DNA fingerprinting with an AmpFlSTR Identifiler PCR Amplification kit (Applied Biosystems #4322288) according to the manufacturer's recommendations. Cells were cultured in RPMI supplemented with 10% fetal bovine serum at 37°C in a humidified 5% CO_2 incubator. HCT116 p53$^{-/-}$ and HCT116 p53$^{+/+}$ and H460 p53-knockdown **cells were treated for 24 hours with 10 µM nutlin 3 (Sigma) to stabilize p53 via MDM2 inhibition. To induce p53 expression in H1299-p53 cells, H1299-p53 cells were treated with 5 µM** ponasterone A (Sigma).

Establishment of Stable p53–Knockdown Cells

[0113] The vectors pRS-shRNAp53 and an shRNA scramble control (from Open Biosystems GE Healthcare Dharmacon Inc.) were transiently transfected with a pPACKH1 HIV Lentivector Packaging Kit (System Biosciences) into 293TN cells using Lipofectamine 2000 and Plus reagents (both from Life Technologies, Carlsbad, CA, USA). Viral supernatant was collected 3 days after transfection and mixed with PEG-it Virus Precipitation Solution (System Biosciences) overnight at 4°C. H460 cells were infected and incubated with the viral particles supplemented with TransDux (System Biosciences) overnight at 37°C. Puromycin (1 µg/mL) was used to select and maintain p53-knockdown in H460 cells. Stable repression of p53 was verified by western blotting.

Target Prediction Databases

[0114] Binding sites for miR-34a in the *PD-L1* 3' UTR were identified by using the target prediction databases miRNA body map (http://www.mirnabodymap.org/; Ghent University, Belgium). These databases compare predicted targets from mirBase release 14, TargetScan 5.1, miRDB 3.0, MicroCosm v5, DIANA 3.0, TarBase v.5c, PITA catalog v6, RNA22 (August 2007), and miRecords v2.

Transfection

[0115] Pre-miR-34a and negative controls (scrambled oligos) (Life Technologies) were reverse-transfected into lung cancer cell lines with Lipofectamine 2000 (Life Technologies) at a final concentration of 100 nM.

Quantitative Polymerase Chain Reaction

[0116] Total RNA was isolated from cells with Triazol (Life Technologies) for miRNA analysis according to the manufacturer's protocol. To analyze expression of mature miR-34a, total RNA was reverse-transcribed using miRNA-specific primers and the TaqMan MicroRNA Reverse Transcription kit from Life Technologies, followed by quantitative polymerase chain reaction (qPCR) with Taqman MicroRNA assays according to the manufacturer's protocol. Relative abundance of miRNA versus U6 expression was calculated by the comparative Ct method.

Protein Extraction and Western Blot Analysis

[0117] Total protein was extracted as previously described [Park B, Yee C, Lee KM. The effect of radiation on the immune response to cancers. Int J Mol Sci 2014;15(1):927-43; Cortez et al., 2014, Mol Ther]. Membranes were probed with primary antibodies directed against PD-L1 (Pierce-Thermo Fisher Scientific, Rockford, IL, USA), p53, GAPDH (Cell Signaling Technologies, Beverly, MA, USA), and a secondary antibody conjugated with horseradish peroxidase (Amersham GE Healthcare). The secondary antibody was visualized by using a chemiluminescent reagent (Pierce ECL kit, Thermo Fisher Scientific, Waltham, MA, USA).

Luciferase Assay

[0118] H1299 cells were seeded into 96-well dishes at 4×10^4 cells/well. Cells were transfected with miR-34a mimics or a scrambled control miRNA (100 nM) together with expression vectors encoding the luciferase gene fused to the *PD-L1* 3′ UTR that is either wild type [wt] or contains a mutated miR-34 binding site (5-bp deletion; SwitchGear Genomics, Carlsbad, CA, USA). Forty-eight hours after transfection, cells were incubated for 30 min with 100 μL/well of LightSwitch Luciferase Assay Reagent (SwitchGear Genomics). Firefly luciferase activity was measured sequentially in luciferase assays (SwitchGear Genomics) using a Fluostar Optima plate reader (BMG Lab Technologies GmbH, Inc, Durham, NC, USA). Three independent experiments were performed, and values are shown as means ± standard error of the mean (SEM).

Chromogenic in Situ Hybridization for miR-34a

[0119] Formalin-fixed paraffin-embedded (FFPE) NSCLC tissue samples, 4–5 μm thick, from patients were mounted on Histogrip-treated microscope slides, dried at 37°C, and baked for 2–4 hours at 60°C. The slides were deparaffinized at 80°C for 30 minutes and then hydrated by dipping the slides into xylene, ethanol and phosphate buffered saline (PBS). A 15 μg/mL solution of pre-

warmed (37°C) proteinase-K (miRCURY LNA microRNA ISH Buffer Set; Exiqon; cat# 90000) was applied and slides were incubated at 37°C for 20 minutes. Then, slides were dehydrated and dried. Twenty-five microliters of 40 nM denatured double-DIG-labeled miRCURY miR-34a LNA probe (Exiqon; cat# 38487-15) diluted in 1X ISH Buffer (miRCURY LNA microRNA ISH Buffer Set; Exiqon; cat# 90000) was added to the center of each sample along with coverslips, and slides were incubated at 53°C for 1 hour. Specificity washes were performed for 5 minutes each at 53°C using 5 x SSC, 1 x SSC, 1 x SSC, 0.2 x SSC, 0.2 x SSC plus one wash with 0.2 x SSC at room temperature (Invitrogen, cat# 15575-038). Tissues were blocked for 15 minutes and incubated for 1 hour at room temperature with anti-DIG antibody alkaline phosphatase (AP) conjugate in a 1:1600 dilution (Roche Life Sciences, cat# 11093274910). After slides were rinsed in PBS-Tween20, freshly prepared AP substrate (NBT/BCIP; Roche Life Sciences) was applied. Slides were incubated for 2 hours at 30°C, protected from light. Tissue slides were rinsed in KTBT (50 mM Tris-HCl, 150 mM NaCl, 10 mM KCl) and nuclease-free water and counterstained with fast red counter stain (American Master Tech; cat# STNFR).

In vivo Tumor Models and Administration of miR-34a/Liposome Complexes

[0120] All mouse studies were approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Texas MD Anderson Cancer Center before their initiation; animal care was provided according to IACUC standards, and all mice had been bred and were maintained in a pathogen-free mouse colony. To create the tumors, syngeneic male 129/Sv mice three to four months of age were injected subcutaneously in the right flank with 10^6 344SQ murine lung adenocarcinoma cells (a lung cancer cell line derived from a spontaneous subcutaneous metastatic lesion in p53R172HΔg/+K-rasLA1/+ mice) (12 mice per group). When tumors reached a volume of approximately 95 mm^3 (range 50–139 mm^3), mice were randomly assigned to one of the following groups such that each group has the same average tumor volume (12 mice per group): Control (no treatment); MRX34 (administered peritumorally three times a week at a dose level of 1 mg per kg mouse bodyweight; 8 total injections; Mirna Therapeutics, Austin, TX); radiotherapy (RT; 6 Gy once a day for 3 days, total 18 Gy); or MRX34 + RT (dose levels and regimens as described above). For the combination therapy group, MRX34 was given 1-2 hours before RT. Mice were immobilized in a jig, and tumors centered within a 3-cm-diameter circle were irradiated using a ^{137}Cesium device (dose rate 4 Gy/min). Tumor growth was assessed 2–3 times a week by measuring tumor volumes using a digital caliper and the formula $V = \text{length} \times \text{width}^2/2$ whereby length is greater than width. Twenty-four hours after the final injection of MRX34, two mice per group were sacrificed. Tumor tissue was collected for immunohistochemical and RNA/protein

analyses to evaluate miR-34a and PD-L1 mRNA and protein expression levels. For PD-L1 expression, specific Taqman probes (Life Technology) were used.

Immunohistochemical Analysis of PD-L1 and p53

[0121] Formalin-fixed patient samples and mouse tissues were processed in an automatic tissue processor, embedded in paraffin (Peloris, Leica) and cut into 4-μm sections. Immunohistochemical staining was done in an automated staining system (Leica Bond Max, Leica Microsystems, Vista, CA, USA). Briefly, slides were deparaffinized and hydrated and antigen was retrieved by incubating in citrate buffer, pH 6.0 for 1 hour with PD-L1 (Thermo Fisher Scientific, #PA5-20343, dilution 1:100) or p53 antibodies (Abcam, #ab4060, dilution 1:100). Staining was microscopically evaluated and graded based on percentage of PD-L1-positive cells as follows: 0=undetectable, 1=1%-25%, 2=26%-50%, 3=51%-75% and 4=76%-100%. The intensity of cellular staining was also graded as undetectable (0), minimal (1), mild (2), moderate (3) or marked (4).

Isolation of Tumor-Infiltrating T Cells, Macrophages, Dendritic Cells, and Myeloid-Derived Suppressor Cells

[0122] Twenty-four hours after the last injection of MRX34, freshly isolated primary tumor tissues (4 mice/group) were washed with ice-cold PBS and digested in PBS supplemented with 3 mg/mL dispase II and 2 mg/mL collagenase at 37°C for 1 hour. Single-cell suspensions were prepared by filtering the digested tissues through 70-μm-pore cell strainers; erythrocytes were removed with red blood cell lysis buffer, and then single-cell suspensions were used for surface or intracellular flow cytometry staining. CD45 staining was used to distinguish immune cells from non-immune cells (tumor and other stromal cells) in tumor tissues, and cells quantified were $CD8^+$ T cells, $CD4^+$ T cells, macrophages (F4/80), myeloid-derived suppressor cells (MDSCs) ($CD11b^+Gr1^+$) and T-regulatory T cells (Tregs) ($CD4^+CD25^+Foxp3^+$). Concomitantly, tumor cells were collected for PD-L1 analysis. Samples were analyzed with an LSRII Flow Cytometer (BD) and data analyzed with FlowJo Software (ThreeStar). Isotype control was used as negative control. The expression of each marker in an untreated control group was considered the basal expression level.

Serum cytokine analysis. Assays for TNFα and IFNγ

[0123] Twenty-four hours after the last injection of MRX34, whole blood samples were collected by cardiac puncture and mixed in EDTA coating tubes from control, MRX34, RT and MRX34+RT treatment groups (4 mice/group). The samples were centrifuged at 1,000 *g* for 10 min. Serum was collected and diluted 1:4 with diluent solution from BioPlex Multiplex assay (Biorad). TNFα and IFNγ were measured by ELISA according to the manufacturer's protocol.

Statistical Analysis

[0124] All data were graphed and analyzed for statistical significance using the Graph Pad (GraphPad Prism, USA),Excel software (Microsoft), or R (version 3.0.1, http:///www.r-project.org/), and P values of less than 0.05 were considered statistically significant. *P* values were generated by two-tailed Student's *t* tests. Data from The Cancer Genome Atlas for Lung Adenocarcinoma (TCGA LUAD) were publicly available and downloaded from TCGA (http://tcga-data.nci.nih.gov/). Level 3 Illumina RNASeqv2 and miRNASeq were used to analyze mRNA and miRNA expression, respectively. For the miRNASeq data, "reads_per_million_miRNA_mapped" values were derived from the "isoform_quantification" files to calculate mature forms for each miRNA. Somatic mutation data from the LUAD samples were downloaded from cBioPortal (http://www.cbioportal.org/). The Spearman's rank-order correlation test was applied to measure the strength of the association between p53 and PD-L1 (CD274) mRNA levels. CD274 levels were compared between p53 mutant tumors and p53 wt tumors with Mann–Whitney tests, as miR-34a levels were not normally distributed ($P<0.05$ from the Shapiro Wilk normality test). miR-34a levels were compared between p53 mutant tumors and p53 wt tumors with *t* tests, as miR-34a levels were normally distributed ($P>0.05$ from the Shapiro Wilk normality test). A box-and-whisker plot (in which the box plot represents the first (lower bound) and the third (upper bound) quartiles, and the whiskers represent 1.5 times the interquartile range) was used to visualize data for these comparisons (log2(x+1)). For each gene/miRNA of interest, their potential relationship with survival was analyzed as follows. Patients were first grouped into percentiles according to mRNA/miRNA expression. Log-rank tests were used to identify associations between mRNA/miRNA expression and overall survival, and the Kaplan-Meier method was used to generate survival curves. Cut-off points that split the samples into low/high mRNA groups (log-rank test P<0.05) were recorded, and cut-offs were also considered for combinations of more than one type of mRNA.

Results

P53 Regulates PD-L1

[0125] In order to investigate the role of p53 in PD-L1 regulation, three different cell systems were used to determine whether the specific induction or depletion of p53 affects PD-L1 expression: (1) isogenic HCT116 $p53^{-/-}$ and $p53^{+/+}$ cells treated with the p53 stabilizer nutlin 3, (2) p53-inducible H1299 cells treated with ponasterone A (PoA), and (3) H460 lung cancer cells transfected with a p53-specific or a scrambled shRNA. p53 expression or lack thereof was confirmed by western blotting (FIGS. 1B, 1D, 1F). Previous studies have shown that p53 operates via miRNAs to

regulate several signaling pathways in cancer. miR-34 family members are well-characterized effector molecules that are transcriptionally induced by p53 and p53 regulates tumor cell recognition by NK cells via miR-34a. Upon testing, miR-34a, miR-34b, and mi-R-34c were expressed at elevated levels in cells that express wild-type p53 (HCT116 $p53^{+/+}$, p53-inducible H1299 treated with PoA, H460 transfected with scr shRNA) relative to their controls (HCT116 $p53^{-/-}$ and p53-inducible H1299 in the absence of PoA), (FIGS. 1A, 1C). In the HCT116 colon cancer cells, levels of miR-34b and miR-34c were higher than levels of miR-34a. On the other hand, in H1299-p53 cells induction of miR-34a was higher than induction of miR-34b and miR-34c (P=0.0001, P < 0.0001, P<0.001 vs P=0.001, P=0.004, P=0.09). In H460+p53 shRNA cells, some downregulation of miR-34b and miR-34c expression was noted relative to H460+scr shRNA, but the decrease was statistically significant only for miR-34a (P = 0.006, P=0.019, and P=0.20) (FIG. 1E). In contrast, PD-L1 was lost or expressed at reduced levels in cells that expressed wild-type p53, suggesting that induction of p53 promoted the downregulation of PD-L1 relative to controls (FIGS. 1B, 1D, 1F). To confirm this inverse relationship of miR-34a and PD-L1 expression *in vivo*, p53-wt and p53-mutated (R175) NSCLC patient samples were used. PD-L1 protein levels were assessed by immunohistochemistry, and miR-34a levels were evaluated by chromogenic in situ hybridization (CISH). NSCLC tumors with mutated p53 had low miR-34a and high PD-L1 levels compared to tumors with wt p53 (FIG. 1G). This result agrees with data from cultured cancer cells and suggests that PD-L1 overexpression in p53-mutated cancers may be relevant to tumor physiology.

P53 expression is inversely correlated with PD-L1 in patients with non-small cell lung cancer (NSCLC)

[0126] The correlation between p53 expression and PD-L1 (CD274) in TCGA samples from 181 patients with NSCLC were analyzed. First, the mRNA expression levels of p53 and PD-L1 (CD274) were compared to analogous mRNAin another study [Stark AM, Pfannenschmidt S, Tscheslog H, et al. Reduced mRNA and protein expression of BCL-2 versus decreased mRNA and increased protein expression of BAX in breast cancer brain metastases: a real-time PCR and immunohistochemical evaluation. Neurol Res 2006;28(8):787-93]. A significant inverse correlation between p53 and PD-L1 (FIG. 2A) (P < 0.001) was found.

[0127] A second analysis comparing PD-L1 expression in NSCLC tumors with mutated p53 (n=84) versus wt p53 (n=97) revealed that mutated p53 tumors had significantly higher PD-L1 levels than wt p53 tumors (FIG. 2B) (P = 0.0294). Accordingly, miR-34a levels were significantly lower in patients with mutated vs. wt p53 (FIG. 2C) (P = 0.0103).

[0128] Patients were stratified based on the type of p53 mutation (functional, partially functional, or nonfunctional mutations) by using the IARC TP53 Database (http://p53.iarc.fr/TP53GeneVariations.aspx) and analyzed expression of miR-34a and PD-L1. No statistically significant differences in PD-L1 among the subgroups was found (P=0.32), although patients with partially functional or nonfunctional p53 seemed to have higher expression of PD-L1 than did patients with wt or functional p53. There was a marginally statistically significant difference in miR-34a levels among these subgroups (P=0.046).

[0129] To check for possible relationships between survival and p53, PD-L1, and miR-34a, the TCGA NSCLC patient data were first grouped into percentiles according to mRNA/miRNA expression and determined that the best cut-off point for low/high p53 was 0.39. For low/high PD-L1 (miR-34a), the cut-off value was 0.69 (P=0.05). It was explored whether combining expression of the two factors (p53 and PD-L1) would improve separation between groups. The the p53/PD-L1 pair was contrasted against the two groups linked to a negative association: high p53/low PD-L1 and low p53/high PD-L1, and found that the best separation occurred at a cutoff of 0.69 for PD-L1 and 0.28 for p53 (P = 0.005) (FIG. 2D). The difference in survival between these two groups was greater than the difference between groups considered based on p53 expression alone (P = 0.03) (FIG. 2F). The p53/miR-34a pair was contrasted against the two groups linked to a positive association: high p53/high miR-34a and low p53/low miR-34a. The best separation was obtained for miR-34a at a cutoff of 0.32 and for p53 at a cutoff of 0.28 (P= 0.004) (FIG. 2E). This result was also an improvement over the results obtained for p53 alone (FIG. 2F).

miR-34a Directly Represses PD-L1 in NSCLC cell lines

[0130] The inverse correlation of miR-34 and PD-L1 expression in cell and tumor samples implicates miR-34 as a regulator downstream of p53 to repress PD-L1. This hypothesis is further corroborated by the observation that the 3'UTR of the PD-L1 mRNA carries a putative miR-34 binding site at position 932-938.

[0131] First, endogenous levels of miR-34a, miR-34b, and miR-34c in NSCLC cell lines with different genetic backgrounds was analyzed (FIG. 3A). miR-34a expression levels were higher than miR-34b and miR-34c levels in all three NSCLC cell lines.

[0132] To test the miR-34-induced repression of PD-L1 experimentally, PD-L1 expression in A549, H460, and H1299 cells transfected with miR-34a was analyzed by western blotting. As shown in FIGS. 3B-G, enforced overexpression of miR-34a suppressed the expression of PD-L1 protein compared with a scrambled control. In addition, enforced overexpression of miR-34b or miR-34c suppressed the expression of PD-L1 protein compared with a scrambled control (FIGS. 3H-J). To determine whether miR-34 interacts directly with the putative target gene PD-L1, H1299

were co-transfected with miR-34a, miR-34b, or miR-34c mimics and a reporter vector encoding the luciferase gene that is fused to the 3′ UTR of the PD-L1 gene (luc-PDL1). As shown in FIG. 3K, luciferase activity was reduced in cells transfected with miR-34 and the luc-PD-L1 construct compared with scrambled controls. In contrast, mutation of the predicted miR-34 binding site in the 3'UTR of PD-L1 rescued the luciferase activity, thus confirming that miR-34a, miR-34b, and miR-34c interact directly with the PD-L1 3′ UTR (fold-change luciferase activity, miR-34a mean = 0.50, SD = 0.2, P<0.001; miR=34b mean 0.52, SD= 0.2, P= 0.006; and miR-34c mean = 0.59, SD= 0.14, P = 0.006) (FIG. 3K).

Therapeutic Delivery of miR-34a Represses PD-L1 in vivo

[0133] Next, the effects of miR-34a replacement on PD-L1 expression in a syngeneic mouse model of NSCLC were tested. To this end, MRX34, a liposomal nanoparticle loaded with miR-34a mimics, was administered peritumorally to murine 344SQ tumors grown subcutaneously in mice. In agreement with our *in vitro* data, it was observed that MRX34 treatment led to an increase of miR-34a levels in the tumor and a concurrent downregulation of tumor PD-L1 mRNA and PD-L1 protein as measured by quantitative real-time polymerase chain reaction (qRT-PCR) (FIG. 4A) and Western blotting (FIG. 4C). The miR-34-induced repression of PD-L1 was further confirmed by flow cytometry (P = 0.04) (FIGS. 4B, D) and immunohistochemical staining of 344SQ tumor tissue (FIGS. 4E, 4F). Liposomal delivery of miR-34 mimics also repressed PD-L1 in the subcutaneous H1299 NSCLC xenograft, demonstrating that the miR-34-mediated regulation of PD-L1 in vivo is observed in a human tumor (FIG. 4G, 4H).

Therapeutic Delivery of miR-34a alone or in combination with RT Increases Tumor-Infiltrating CD8+ T Cells and Decreases PD-1+ T cells, and Macrophages

[0134] To explore the effects of MRX34 on tumor growth, the tumor milieu, and its associated immune cells, a multi-dose efficacy study in the 344SQ syngeneic mouse model was designed.

[0135] Subcutaneous tumors were created by inoculating 1 x 10^6 344SQ cells derived from a spontaneous subcutaneous lung metastasis from a p53R172HΔg/+K-rasLA1/+ mouse into the right leg of each syngeneic 129Sv/Ev mouse. One week after tumor implantation, mice were randomly assigned to one of four groups: control; MRX34 only; RT; and MRX34 plus RT. The formulation was given as subcutaneous injections at a dose of 1 mg/kg (total of 8 injections), and local irradiation was given to a total dose of 18 Gy, in 6-Gy fractions given over 3 days starting when tumors were 8 mm in diameter. For the combination-therapy condition, MRX34 was given 1 hour before radiation.

[0136] Because radiation therapy (RT) has been shown to induce adaptive immune responses to promote tumor regression and because it was previously observed that MRX34 enhances the effects

of RT in lung xenograft models when used in combination, the effects of the MRX34 in combination with RT and assigned mice to a combination group and a RT alone group was tested. MRX34 treatment resulted in increased numbers of tumor-infiltrating CD8+ cells (FIGS. 5A, 5B, 5C) (P = 0.016, n=4) and also reduced the numbers of tumor-infiltrating PD-1+ T cells (FIGS. 5F, 5G, 5H) (P = 0.001, n=4), macrophages (P = 0.008, n=4) (FIGS. 5K, 5L, 5M) and Tregs (FIGS. 5P, 5Q, 5R) compared with the control condition, although the latter difference was not statistically significant. RT alone also led to an increase of CD8+ cells (P = 0.02, n=4) (FIGS. 5A, 5B, 5D) and a decrease in PD-1+ cells in the tumor (P = 0.04, n=4) (FIGS. 5F, 5G, 5I); however, and in contrast to MRX34, RT led to an increase of macrophages (FIGS. 5K, 5L, 5N) and Tregs (FIGS. 5P, 5Q, 5S). The combined use of MRX34 and RT resulted in an even greater increase of CD8+ T cells (P = 0.004, n=4) (FIGS. 5A, 5B, 5E) compared to each therapy alone and a decrease in PD-1+ T cells (P = 0.02, n=4) (FIGS. 5B, 5G, 5J). MRX34 in combination with RT also counteracted the effects of RT on macrophages (FIGS. 5K, 5L, 5O) and Tregs (FIGS. 5P, 5Q, 5T), both of which were lower in the combination relative to RT alone. These treatments, alone and in combination, did not affect numbers of dendritic cells and may have slightly increased the numbers of MDSCs. In accordance with the decline in exhausted T cells (PD-1+) and an increase of CD8+ T cells, it was **found that MRX34 and RT produced increases in IFNγ (P = 0.004, n=4) (FIG. 5U) and in TNFα (P = 0.02, n=4) (FIG. 5V) compared with the control conditions; IFNγ levels were also increased in** the MXR34-only condition (P = 0.003, n=4). Finally, MRX34, RT and MRX34 plus RT delayed tumor growth relative to the control group with the combination being the most effective therapy (FIG 5W).

Discussion

[0137] These data defined a new role for p53 and suggested that p53 specifically modulates the tumor immune response via miR-34a and PD-L1. This tied tumor immune evasion to other tumor suppressor pathways previously described for p53 and miR-34a, such as apoptosis, DNA damage and cell cycle, and further implicated p53 and miR-34 in immune cell regulation. For instance, p53 regulates tumor cell recognition by NK cells via miR-34a regulation. miR-34 can also function in a **feedback loop to TGFβ regulating the chemokine CCL22 and tumor immune escape via recruitment of Tregs. Another study showed that miR-34a regulates diacylglycerol kinase ζ (DGKζ), a protein that regulates T-cell activation after engagement of the T cell receptor.**

[0138] Patients with NSCLC that expressed high PD-L1 and low p53 levels had lower survival rates than did patients with low PD-L1/high p53 tumors, and those with high miR-34a/ high p53 had better survival rates than those with low miR-34a/low p53 or simply low p53 expression. These

findings indicated that, in some instances, p53 and PD-L1 expression are useful biomarkers of response to therapy.

[0139] *In vivo* delivery of miR-34 via MRX34 in the syngeneic tumor model increased the number of tumor-infiltrating CD8+ T cells and decreased the number of exhausted CD8+PD-1+ T cells, macrophages and Tregs, suggesting that miR-34 may have a direct impact on immune evasion that can be exploited therapeutically. The effect on CD8+ T cells was augmented in combination with RT that had previously been shown to induce adaptive immune responses. This result also agreed with another study showing that miR-34a promotes T-cell activation by regulating DGKζ and CD69 [Shin J, Xie D, Zhong XP. MicroRNA-34a enhances T cell activation by targeting diacylglycerol kinase zeta. PLoS One 2013;8(10):e77983]. Interestingly, it was found that MRX34 decreased PD-1+ T cells compared with control, RT-only, or combination therapy. In accordance with the decrease in numbers of PD-1+ cells, it was also found that IFNγ and TNFα were increased by MRX34 and RT. These results, in combination with the discovery that MRX34 combined with RT delayed tumor growth in another mouse model, indicated that miR-34a plays a key role in T-cell exhaustion.

[0140] These findings on macrophages and Tregs agreed with those of previous studies showing that miR-34a regulates CSF1R, the receptor for CSF1 expressed by macrophages. In some instances, the use of anti-CSF1R antibodies to target tumor-associated macrophages has potential as an anticancer approach. Interestingly, recent studies showed that reduced miR-34a expression correlated with enhanced recruitment of Tregs via upregulation of CCL22, suggesting a potential role for miR-34a in regulating tumor immune evasion in lung cancer.

[0141] Taken together, these data identify a novel mechanism by which tumor immune evasion is regulated by p53 and miR-34a via PD-L1. In some instances, the therapeutic delivery of miR-34a combined with standard therapies, such as RT, represents a new modality of immunotherapy. In some instances, the ability of miRNAs to target multiple cellular processes provide an advantage over current approaches that are limited to targeting single aspects of immunogenic or other signaling pathways (e.g., antibodies such as ipililumab and nivolumab or small-molecule inhibitors such as EGFR inhibitors).

Example 2: Study to Evaluate the Effectiveness, Safety, and Tolerability of MRX34 and the Combination of MRX34 plus Atezolizumab in Subjects with Bladder Cancer

[0142] The first part of the study is a dose escalation phase designed to establish the safety of MRX34 at different dose levels for individuals with bladder cancer.

[0143] The second part of the study is the expansion phase designed to generate additional clinical data at specific doses and adds a second cohort to compare the efficacy of MRX34 plus

atezolizumab in the treatment of bladder cancer. 1200 mg of Atezolizumab is administered by intravenous infusion on day 1 of each 21 day cycle. MRX34 is administered daily for the first five days of a 21 day cycle. The dosage of MRX34 in the expansion phase is the dosage determined in the dose escalation phase.

[0144] These studies are carried out on 600 individuals 18 years and older, both male and female, with histologically confirmed advanced bladder cancer not eligible for surgical and/or locoregional therapies. Individuals are excluded who have a history of autoimmune disease.

[0145] Primary outcome measures include: 1) safety and tolerability of MRX34 as evaluated by incidence of adverse events, serious adverse events, adverse events leading to discontinuation, deaths, and clinical laboratory test abnormalities; 2) objective response rate during the expansion phase of MRX34; 3) safety and tolerability of MRX34 plus Atezolizumab is evaluated by incidence of adverse events, serious adverse events, adverse events leading to discontinuation, deaths, and clinical laboratory test abnormalities; and 4) objective response rate during MRX34 plus Atezolizumab combination cohort.

Example 3: Clinical Trial of a MRX34 in combination with radiotherapy as a Treatment for Glioblastoma

[0146] The first part of the study is a dose escalation phase designed to establish the safety of MRX34 at different dose levels for individuals with glioblastoma.

[0147] The second part of the study is the expansion phase designed to generate additional clinical data at specific doses and adds a second cohort to compare the efficacy of MRX34 plus radiotherapy in the treatment of glioblastoma. MRX34 is administered daily for the first five days of a 21 day cycle. The dosage of MRX34 in the expansion phase is the dosage determined in the dose escalation phase. Individuals undergo once-a-day (QD) radiation treatments 5 days a week for 6 weeks, the indivdual receiving 60 Gy of radiation. The 21-day cycle of MRX34 repeats for up to 18 months in the absence of disease progression or unacceptable toxicity.

[0148] These studies are carried out on 100 individuals 18 years and older, both male and female, with glioblastoma not eligible for surgical and/or locoregional therapies. Individuals are excluded who have a history of autoimmune disease.

[0149] Primary outcome measures include: 1) safety and tolerability of MRX34 as evaluated by incidence of adverse events, serious adverse events, adverse events leading to discontinuation, deaths, and clinical laboratory test abnormalities; 2) objective response rate during the expansion phase of MRX34; 3) safety and tolerability of MRX34 plus Atezolizumab is evaluated by incidence of adverse events, serious adverse events, adverse events leading to discontinuation, deaths, and

clinical laboratory test abnormalities; and 4) objective response rate during MRX34 plus Atezolizumab combination cohort.

[0150] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating a PD-L1 expressing cancer in an individual in need thereof comprising:
    a. identifying the cancer as a PD-L1 expressing cancer; and
    b. providing a synthetic miR-34 oligonucleotide to the individual.
2. The method of claim 1, further comprising providing a PD-1 or PD-L1 inhibitor to the individual.
3. The method of claim 2, wherein the PD-1 or PD-L1 inhibitor is an antibody, small interfering RNA, or antisense RNA.
4. The method of claim 2, wherein the synthetic miR-34 oligonucleotide and the PD-1 or PD-L1 inhibitor are synergistic.
5. The method of claim 2, wherein the synthetic miR-34 oligonucleotide is provided before the PD-1 or PD-L1 inhibitor.
6. The method of claim 1, further comprising providing radiotherapy to the individual.
7. The method of claim 6, wherein the synthetic miR-34 oligonucleotide is provided before the radiotherapy.
8. The method of claim 1 wherein the cancer is a solid tumor.
9. The method of claim 8, wherein the solid cancer is a melanoma, nasopharyngeal cancer, neuroendrocrine tumor, lung cancer, colon cancer, urothelial cancer, bladder cancer, liver cancer, multiple myeloma, ovarian cancer, gastric carcinoma, esophageal cancer, pancreatic cancer, kidney cancer, breast cancer, or lymphoma.
10. The method of claim 9, wherein the lung cancer is a non-small cell lung cancer (NSCLC).
11. The method of claim 9, wherein the lung cancer is a small-cell lung cancer (SCLC).
12. The method of claim 9, wherein the liver cancer is a hepatocellular carcinoma (HCC).
13. The method of claim 1, wherein the cancer is a leukemia.
14. The method of claim 1, wherein the cancer is a lymphoma.
15. The method of claim 1, wherein identifying the cancer as a PD-L1 expressing cancer comprises measuring the PD-L1 expression in a cancer cell from the individual and comparing to a control.
16. The method of claim 15, wherein the PD-L1 expression is overexpressed compared to the control.
17. The method of claim 1, further comprising selecting the individual having functional p53.
18. The method of claim 1, wherein the synthetic miR-34 oligonucleotide is administered in a liposomal formulation.

19. The method of claim 1, wherein T cell exhaustion is prevented or reduced.
20. The method of claim 1, wherein the synthetic miR-34 oligonucleotide comprises:
    a. an active strand comprising a sequence at least 80% identical to a mature miRNA; and
    b. a separate passenger strand that is at least 60% complementary to the active strand.
21. The method of claim 20, wherein the passenger strand of the synthetic miR-34 oligonucleotide comprises a 5' terminal cap.
22. The method of claim 21, wherein the 5' terminal cap is a lower alkylamine.
23. The method of claim 21, wherein the 5' terminal cap is NH_2-$(CH_2)_6$-O-.
24. The method of claim 20, wherein the mature miRNA comprises: miR-34a (SEQ ID NO:1), miR-34b (SEQ ID NO:2), miR-34c (SEQ ID NO:3), or a miR-34 consensus sequence (SEQ ID NO:4).
25. The method of claim 20, wherein the mature miRNA comprises: miR-449a (SEQ ID NO:5), miR-449b (SEQ ID NO:6), miR-449c (SEQ ID NO:7), or a miR-449 consensus sequence (SEQ ID NO:8).
26. The method of claim 20, wherein the mature miRNA comprises a miR-34/449 seed sequence (SEQ ID NO:9).
27. A method of treating a PD-L1 expressing cancer in an individual in need thereof comprising:
a. providing a synthetic miR-34 oligonucleotide to the individual; and
b. providing a PD-1 or PD-L1 inhibitor to the individual.
28. The method of claim 27, wherein the PD-1 or PD-L1 inhibitor is an antibody, small interfering RNA, or antisense RNA.
29. The method of claim 27 wherein the synthetic miR-34 oligonucleotide and the PD-1 or PD-L1 inhibitor are synergistic.
30. The method of claim 27, wherein the synthetic miR-34 oligonucleotide is provided before the PD-1 or PD-L1 inhibitor.
31. The method of claim 27, further comprising providing radiotherapy to the individual.
32. The method of claim 31, wherein the synthetic miR-34 oligonucleotide is provided before the radiotherapy.
33. The method of claim 27 wherein the cancer is a solid tumor.
34. The method of claim 33, wherein the solid cancer is a melanoma, nasopharyngeal cancer, neuroendrocrine tumor, lung cancer, colon cancer, urothelial cancer, bladder cancer, liver cancer, multiple myeloma, ovarian cancer, gastric carcinoma, esophageal cancer, pancreatic cancer, kidney cancer, breast cancer, or lymphoma.

35. The method of claim 34, wherein the lung cancer is a non-small cell lung cancer (NSCLC).
36. The method of claim 34, wherein the lung cancer is a small-cell lung cancer (SCLC).
37. The method of claim 34, wherein the liver cancer is a hepatocellular carcinoma (HCC).
38. The method of claim 27, wherein the cancer is a leukemia.
39. The method of claim 27, wherein the cancer is a lymphoma.
40. The method of claim 27, wherein identifying the cancer as a PD-L1 expressing cancer comprises measuring the PD-L1 expression in a cancer cell from the individual and comparing to a control.
41. The method of claim 40, wherein the PD-L1 expression is overexpressed compared to the control.
42. The method of claim 27, further comprising selecting the individual having functional p53.
43. The method of claim 27, wherein the synthetic miR-34 oligonucleotide is administered in a liposomal formulation.
44. The method of claim 27, wherein T cell exhaustion is prevented or reduced.
45. The method of claim 27, wherein the synthetic miR-34 oligonucleotide comprises:
    a. an active strand comprising a sequence at least 80% identical to a mature miRNA; and
    b. a separate passenger strand that is at least 60% complementary to the active strand.
46. The method of claim 45, wherein the passenger strand of the synthetic miR-34 oligonucleotide comprises a 5' terminal cap.
47. The method of claim 46, wherein the 5' terminal cap is a lower alkylamine.
48. The method of claim 46, wherein the 5' terminal cap is NH_2-$(CH_2)_6$-O-.
49. The method of claim 45, wherein the mature miRNA comprises: miR-34a (SEQ ID NO:1), miR-34b (SEQ ID NO:2), miR-34c (SEQ ID NO:3), or a miR-34 consensus sequence (SEQ ID NO:4).
50. The method of claim 45, wherein the mature miRNA comprises: miR-449a (SEQ ID NO:5), miR-449b (SEQ ID NO:6), miR-449c (SEQ ID NO:7), or a miR-449 consensus sequence (SEQ ID NO:8).
51. The method of claim 45, wherein the mature miRNA comprises a miR-34/449 seed sequence (SEQ ID NO:9).
52. A method of treating an individual suffering from a chronic infectious disease comprising:
    a. providing a synthetic miR-34 oligonucleotide to the individual; and
    b. providing a PD-1 or PD-L1 inhibitor to the individual.

53. The method of claim 52, wherein the PD-1 or PD-L1 inhibitor is an antibody, small interfering RNA, or antisense RNA.
54. The method of claim 52 wherein the synthetic miR-34 oligonucleotide and the PD-1 or PD-L1 inhibitor are synergistic.
55. The method of claim 52, wherein the synthetic miR-34 oligonucleotide is provided before the PD-1 or PD-L1 inhibitor.
56. The method of claim 52, wherein the infectious disease is a persistent viral infection.
57. The method of claim 56, wherein the viral infection is human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), rhinovirus (common cold), herpes simplex virus (HSV), or respiratory syncytial virus (RSV).
58. The method of claim 52, wherein the infectious disease is a persistent bacterial infection.
59. The method of claim 58, wherein the bacterial infection is *Helicobacter pylori*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, or *Chlamydia trachomatis*.
60. The method of claim 52, wherein the infectious disease is the result of infection by a parasite, protozoan, or metazoan.
61. The method of claim 60, wherein the parasite, protozoan, or metazoan is *Schistosoma mansoni*, *Taenia crassiceps*, or *Leishmania mexicana*.
62. The method of claim 52, further comprising selecting the individual having functional p53.
63. The method of claim 52, wherein the synthetic miR-34 oligonucleotide is administered in a liposomal formulation.
64. The method of claim 52, wherein T cell exhaustion is prevented or reduced.
65. The method of claim 52, wherein the synthetic miR-34 oligonucleotide comprises:
    a. an active strand comprising a sequence at least 80% identical to a mature miRNA; and
    b. a separate passenger strand that is at least 60% complementary to the active strand.
66. The method of claim 65, wherein the passenger strand of the synthetic miR-34 oligonucleotide comprises a 5’ terminal cap.
67. The method of claim 66, wherein the 5’ terminal cap is a lower alkylamine.
68. The method of claim 66, wherein the 5’ terminal cap is NH_2-$(CH_2)_6$-O-.
69. The method of claim 65, wherein the mature miRNA comprises: miR-34a (SEQ ID NO:1), miR-34b (SEQ ID NO:2), miR-34c (SEQ ID NO:3), or a miR-34 consensus sequence (SEQ ID NO:4).

70. The method of claim 65, wherein the mature miRNA comprises: miR-449a (SEQ ID NO:5), miR-449b (SEQ ID NO:6), miR-449c (SEQ ID NO:7), or a miR-449 consensus sequence (SEQ ID NO:8).
71. The method of claim 65, wherein the mature miRNA comprises a miR-34/449 seed sequence (SEQ ID NO:9).
72. A composition comprising:
    a. a synthetic miR-34 oligonucleotide, comprising:
        i. an active strand comprising a sequence at least 80% identical to a mature miRNA; and
        ii. a separate passenger strand that is at least 60% complementary to the active strand; and
    b. a PD-1 or PD-L1 inhibitor.
73. The composition of claim 72, wherein the passenger strand of the synthetic miR-34 oligonucleotide comprises a 5' terminal cap.
74. The composition of claim 73, wherein the 5' terminal cap is a lower alkylamine.
75. The composition of claim 73, wherein the 5' terminal cap is NH_2-$(CH_2)_6$-O-.
76. The composition of claim 72, wherein the mature miRNA comprises: miR-34a (SEQ ID NO:1), miR-34b (SEQ ID NO:2), miR-34c (SEQ ID NO:3), or a miR-34 consensus sequence (SEQ ID NO:4).
77. The composition of claim 72, wherein the mature miRNA comprises: miR-449a (SEQ ID NO:5), miR-449b (SEQ ID NO:6), miR-449c (SEQ ID NO:7), or a miR-449 consensus sequence (SEQ ID NO:8).
78. The composition of claim 72, wherein the mature miRNA comprises a miR-34/449 seed sequence (SEQ ID NO:9).
79. The method of claim 72, wherein the PD-1 or PD-L1 inhibitor is an antibody, small interfering RNA, or antisense RNA.
80. The method of claim 72, wherein the synthetic miR-34 oligonucleotide and the PD-1 or PD-L1 inhibitor are synergistic.
81. The method of claim 72, wherein the synthetic miR-34 oligonucleotide is provided before the PD-1 or PD-L1 inhibitor.

FIG. 1A

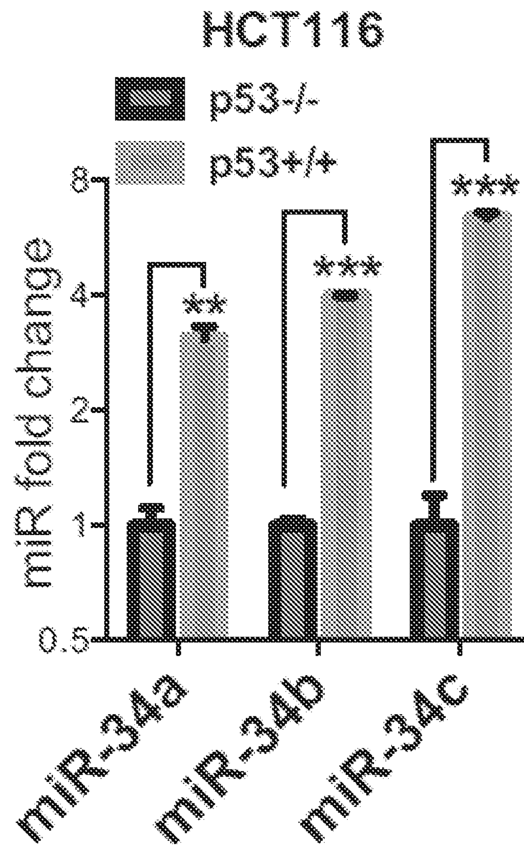


FIG. 1B

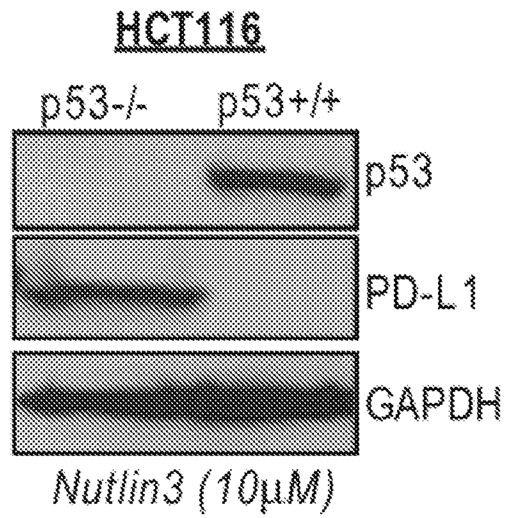


FIG. 1C

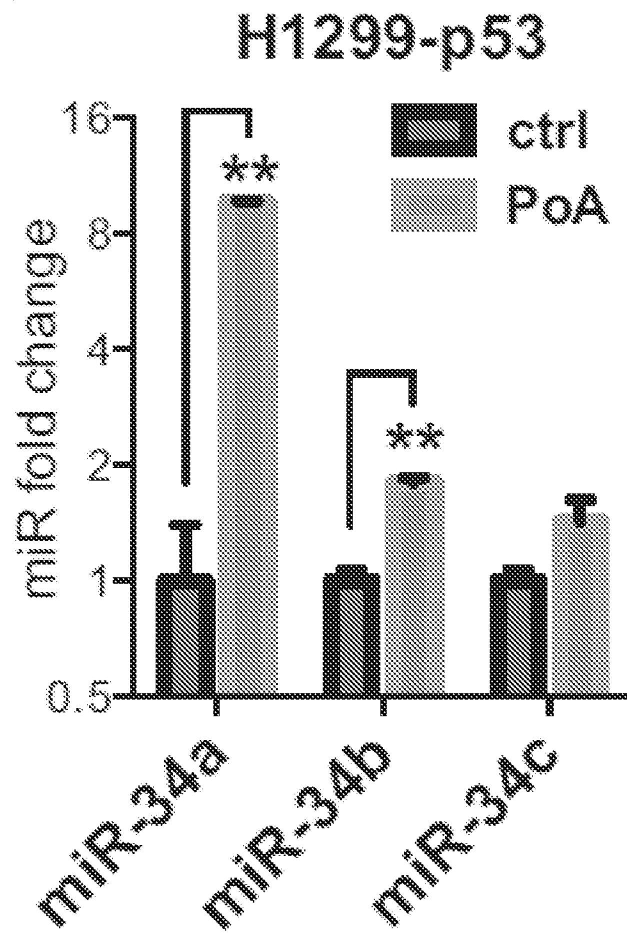


FIG. 1D

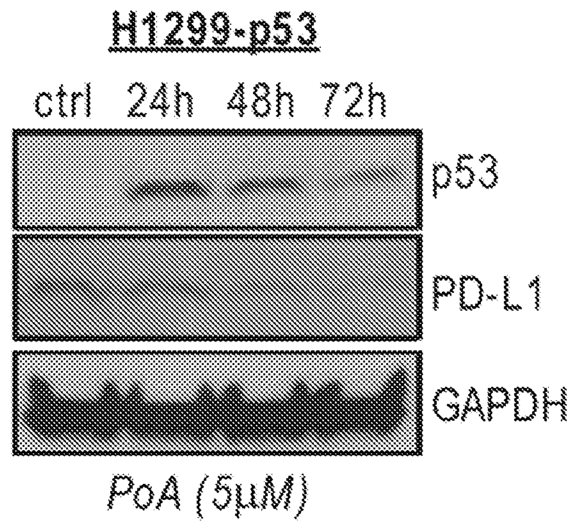


FIG. 1E

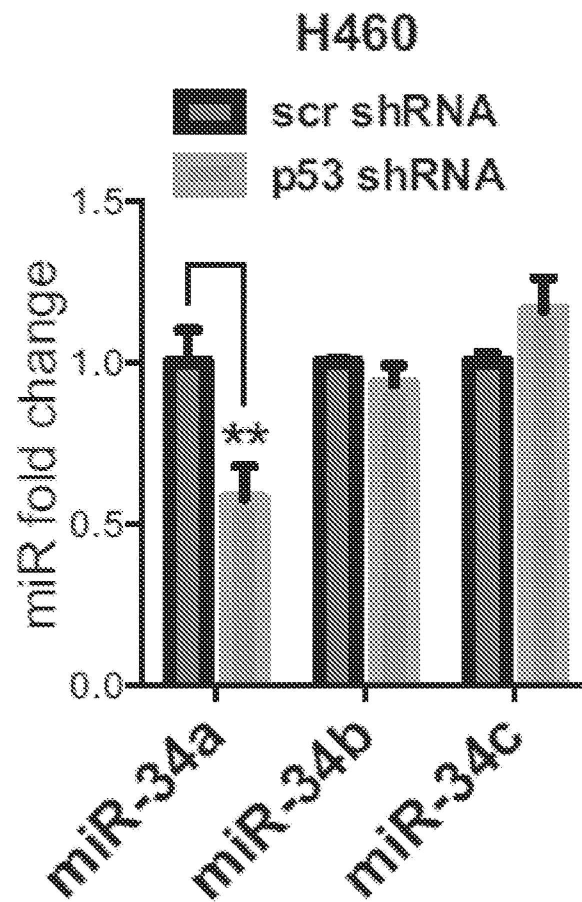


FIG. 1F

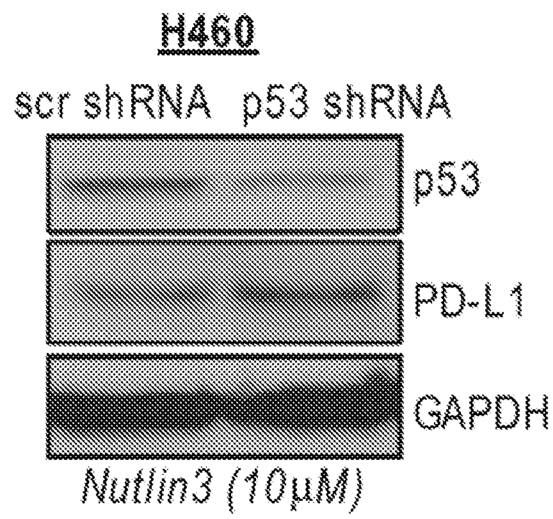


FIG. 1G

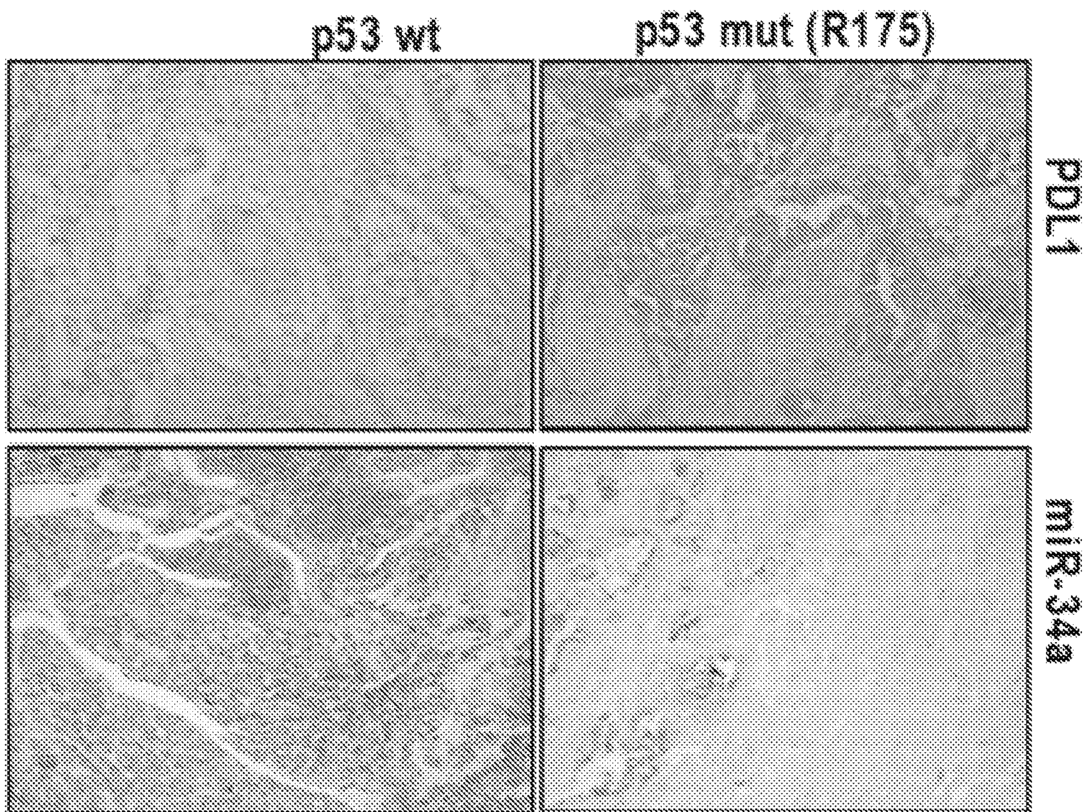


FIG. 2A

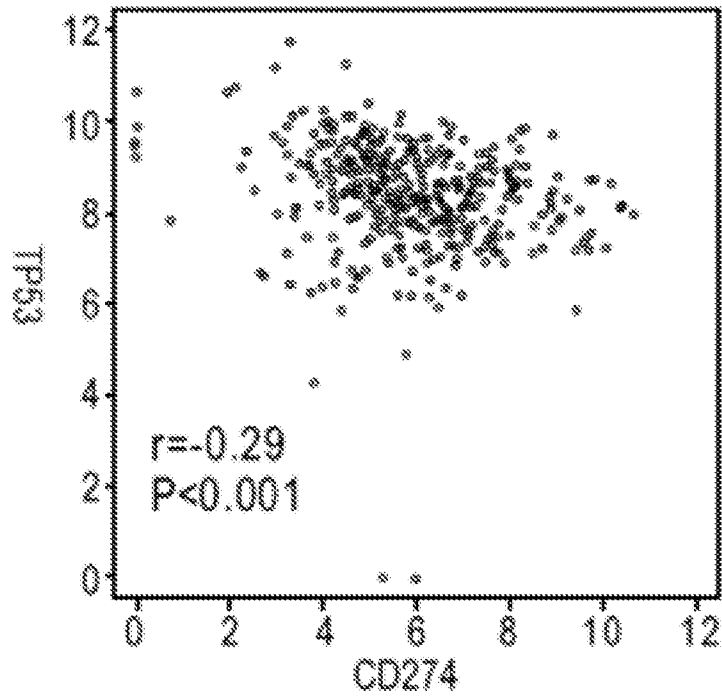


FIG. 2B

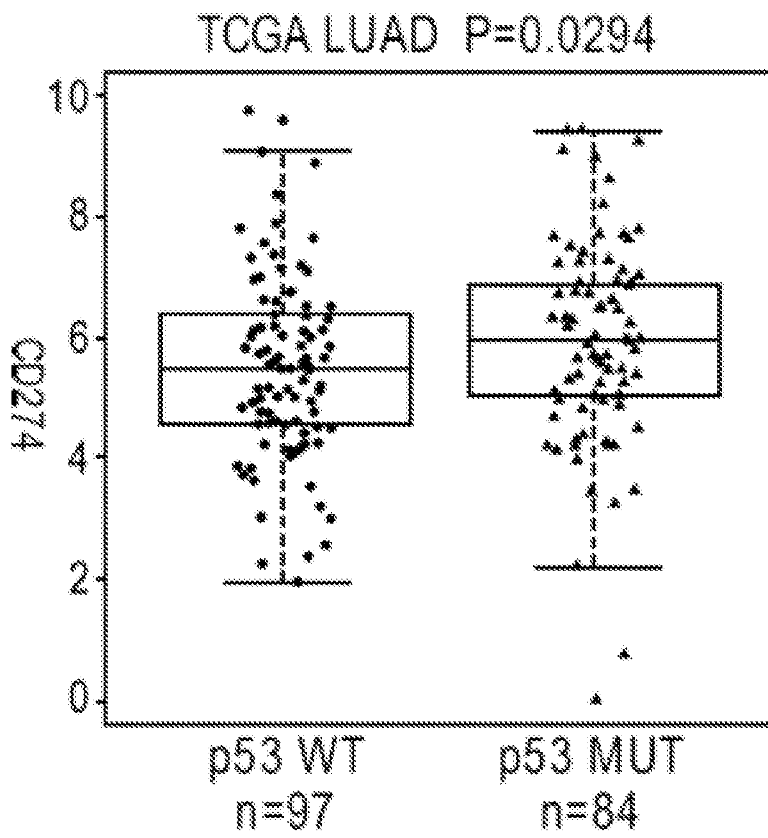


FIG. 2C

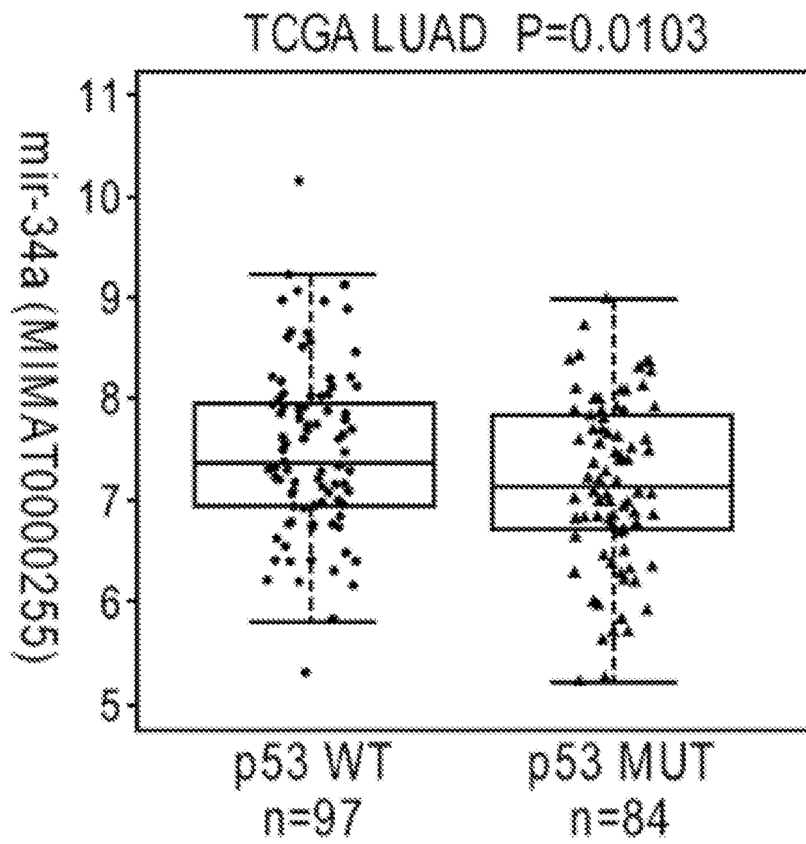


FIG. 2D

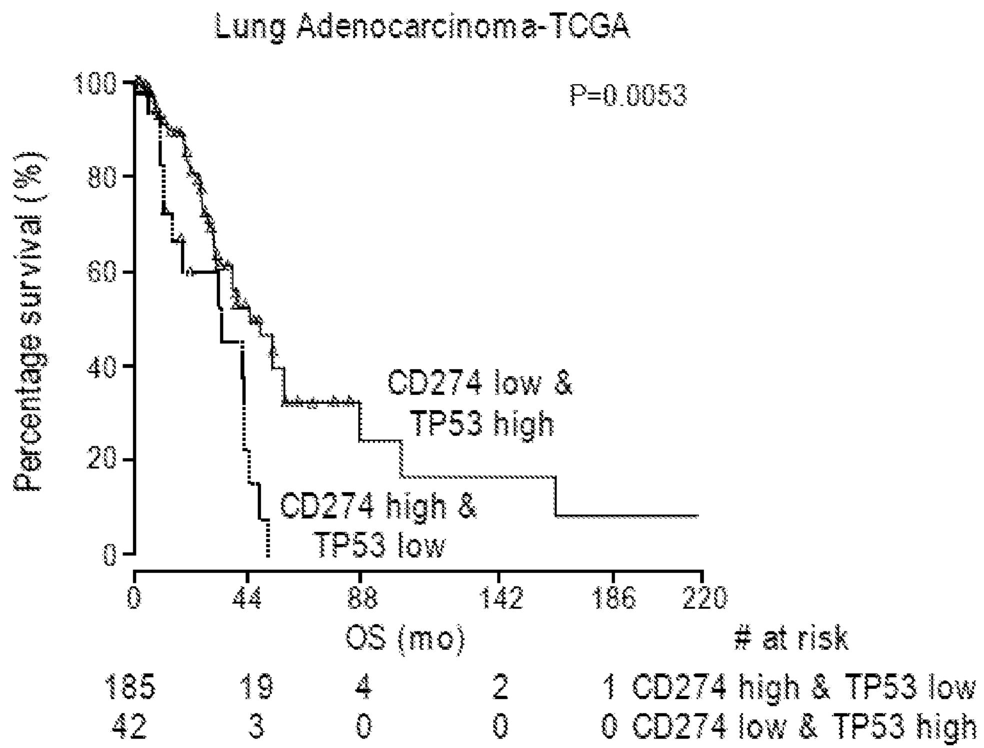


FIG. 2E

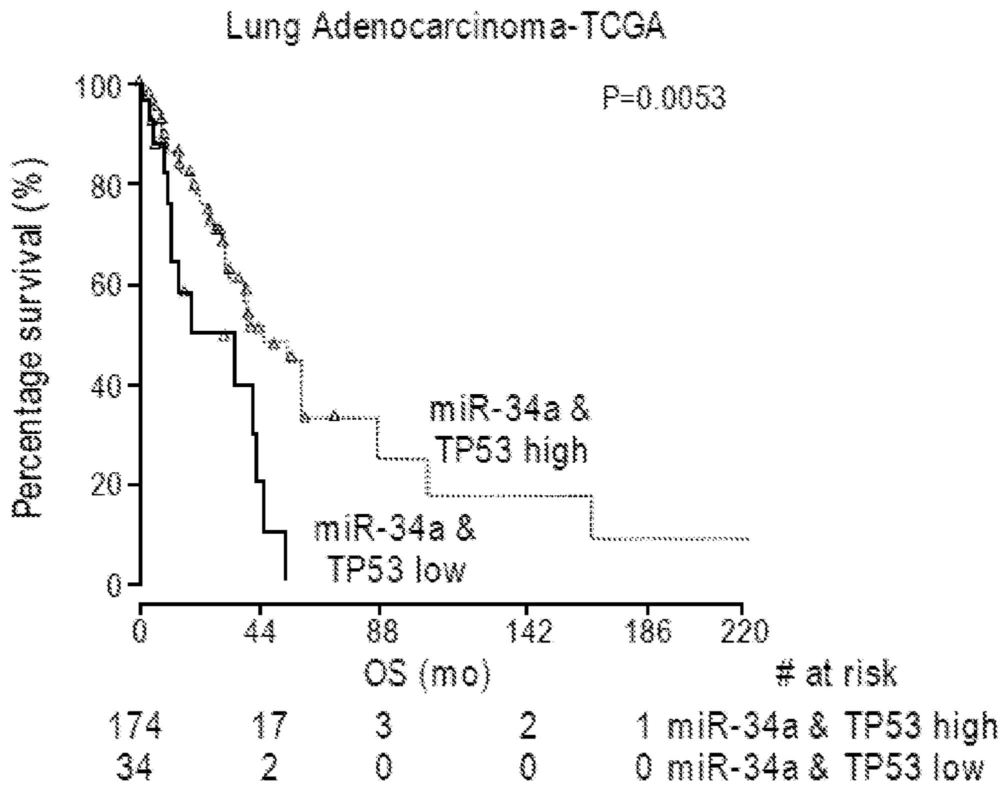


FIG. 2F

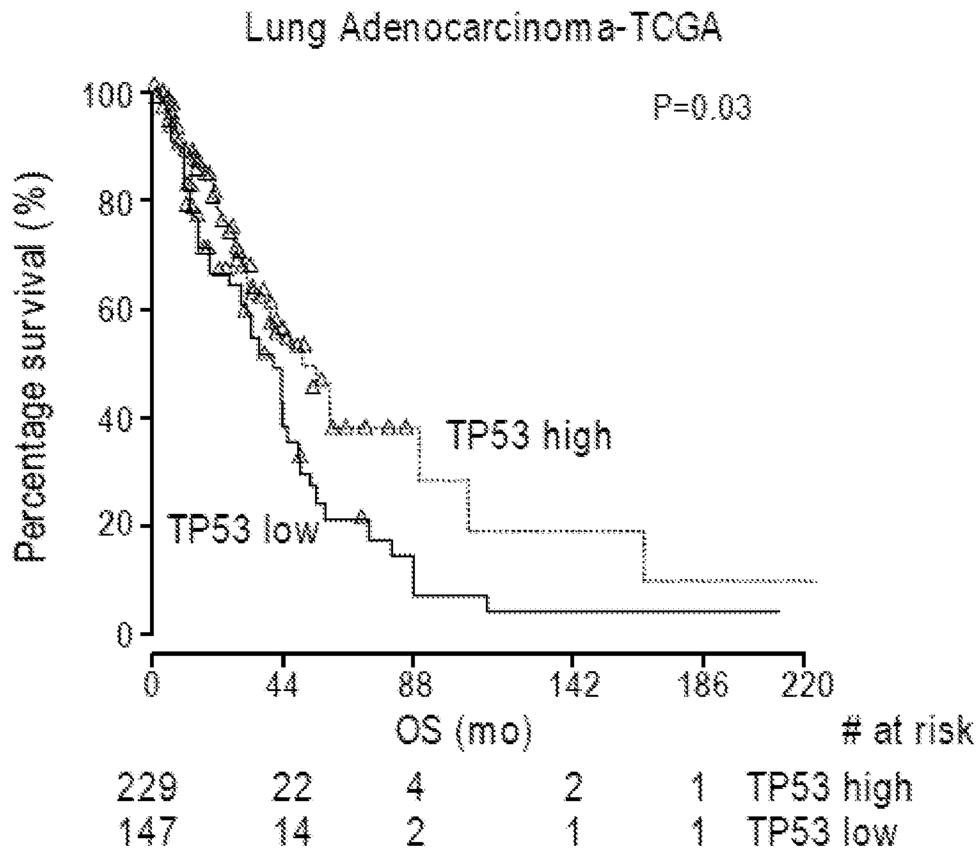


FIG. 3A

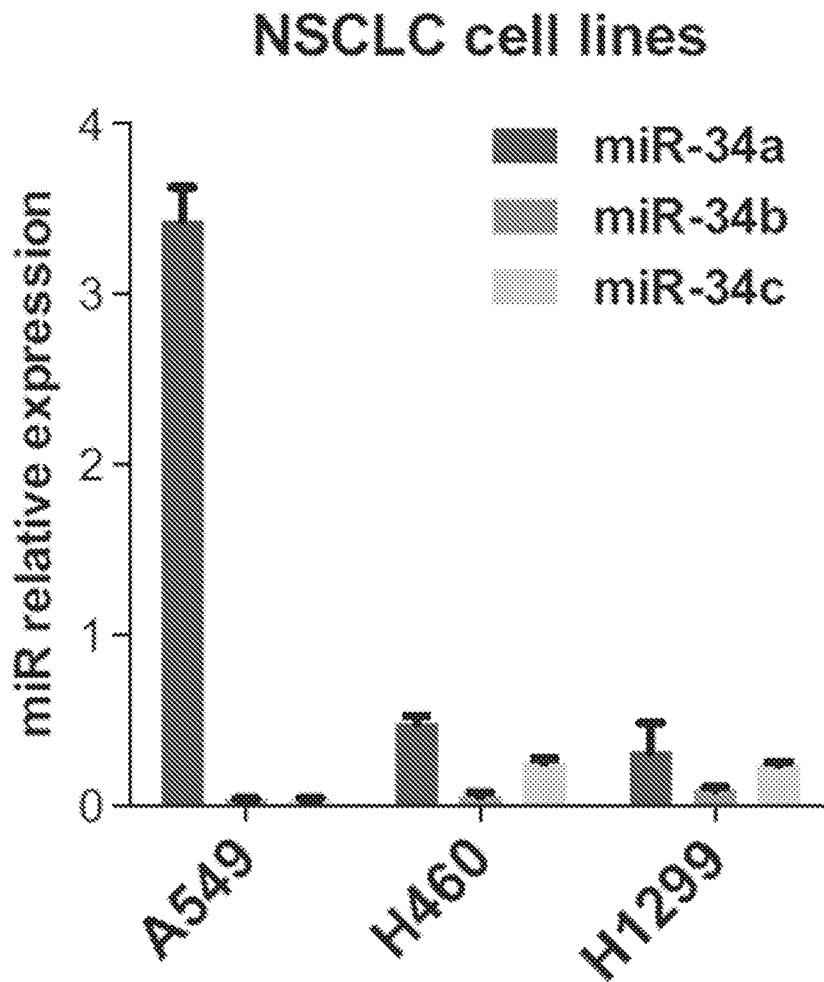


FIG. 3B

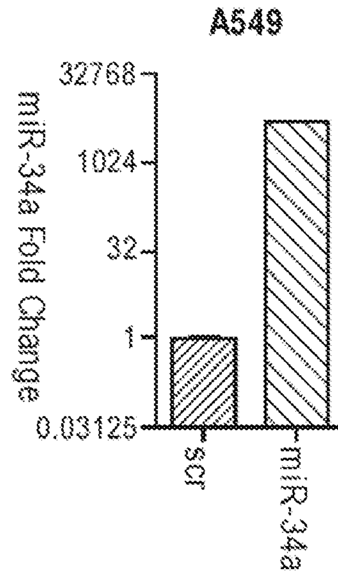


FIG. 3C

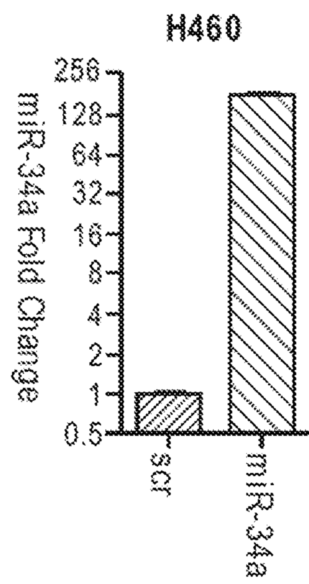


FIG. 3D

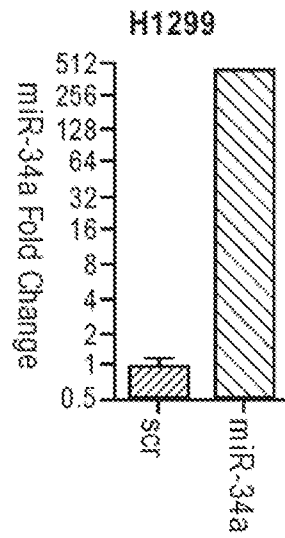


FIG. 3E

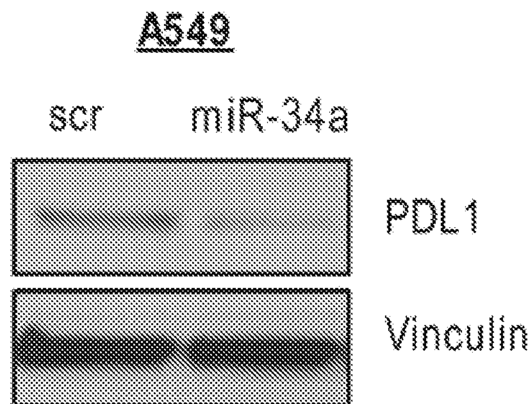


FIG. 3F

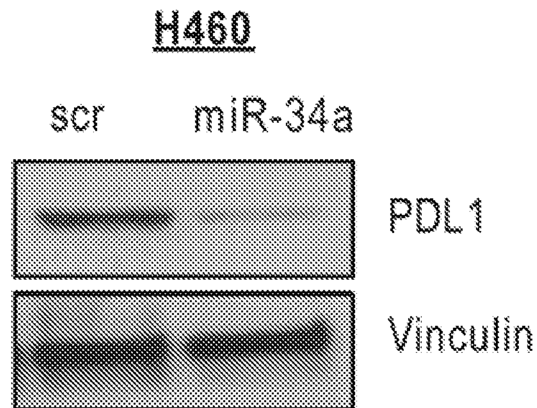


FIG. 3G

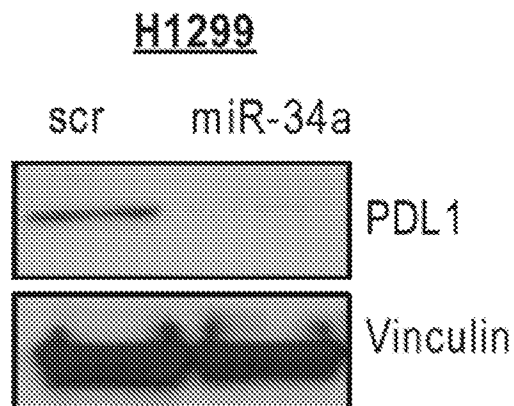


FIG. 3H

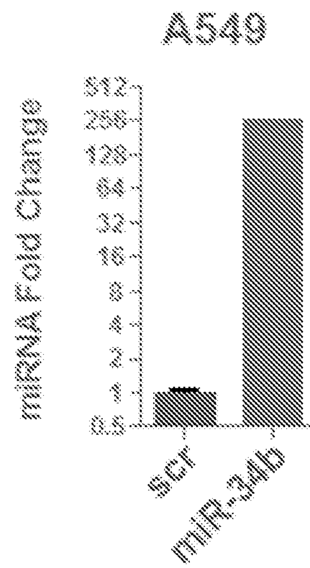


FIG. 3I

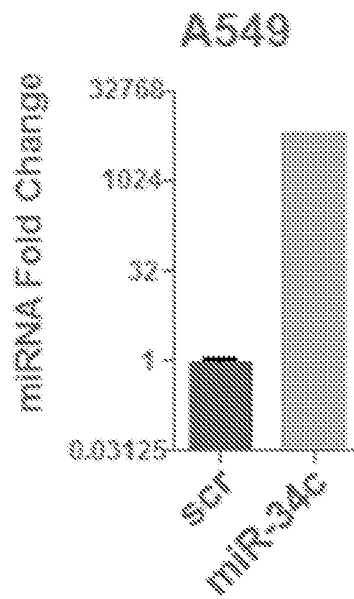


FIG. 3J

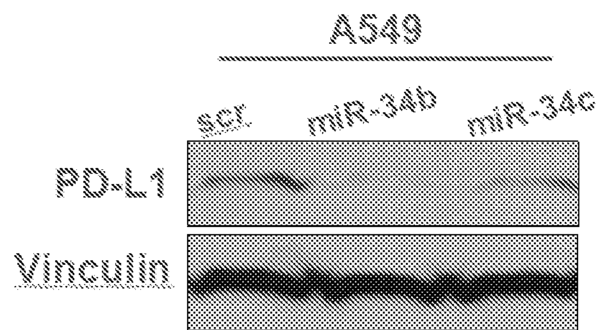


FIG. 3K

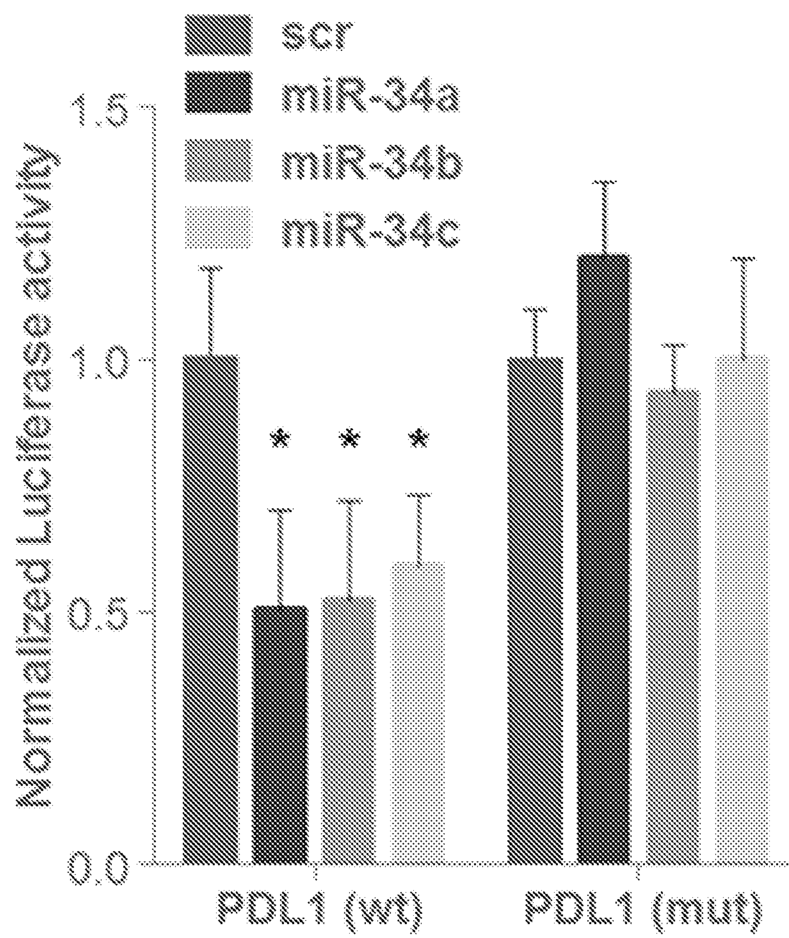


FIG. 3L

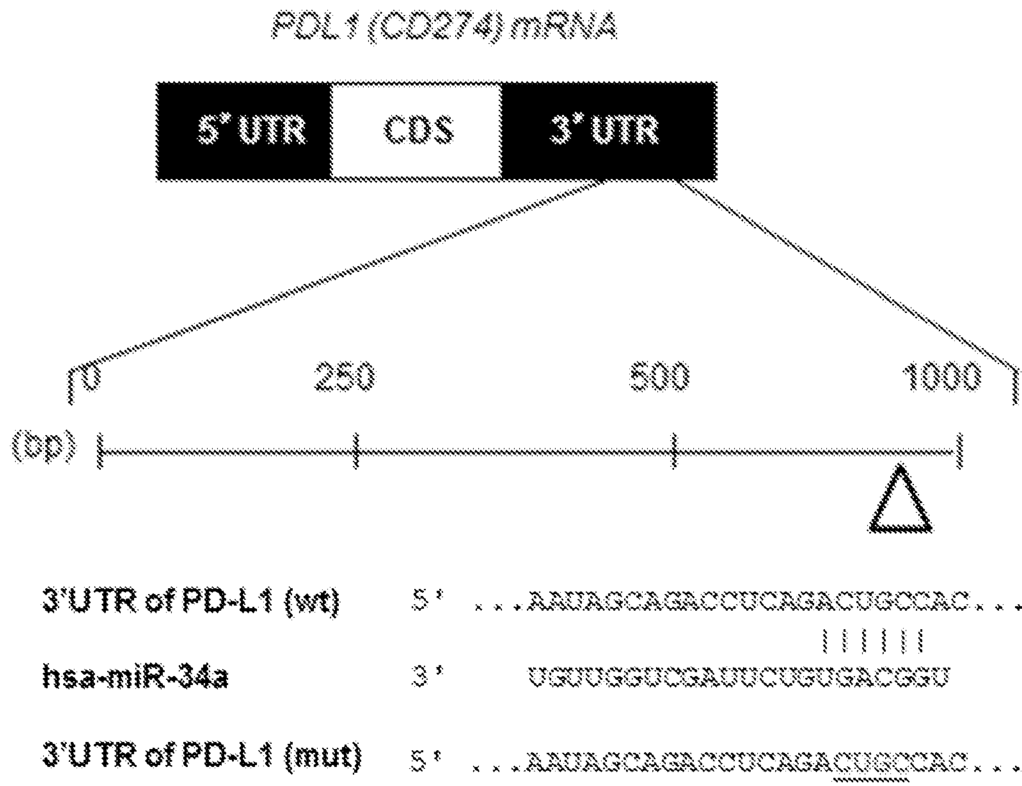


FIG. 4A

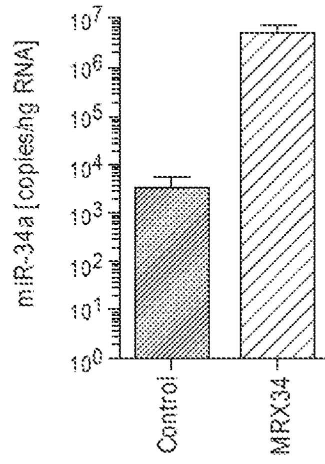


FIG. 4B

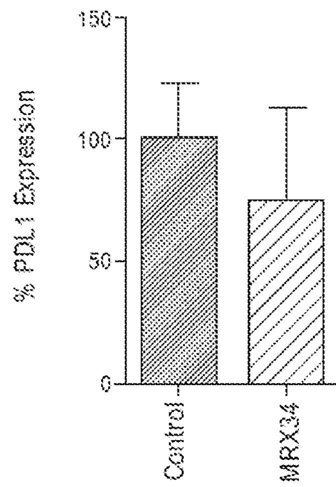


FIG. 4C

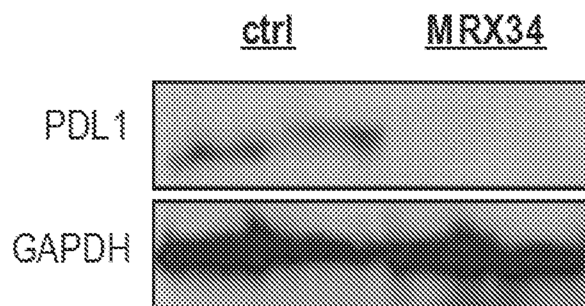


FIG. 4D

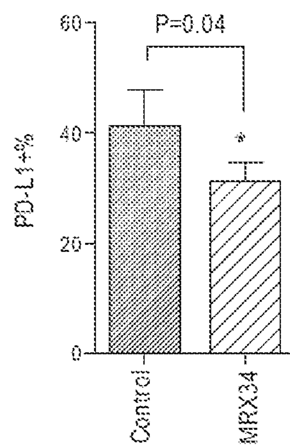


FIG. 4E

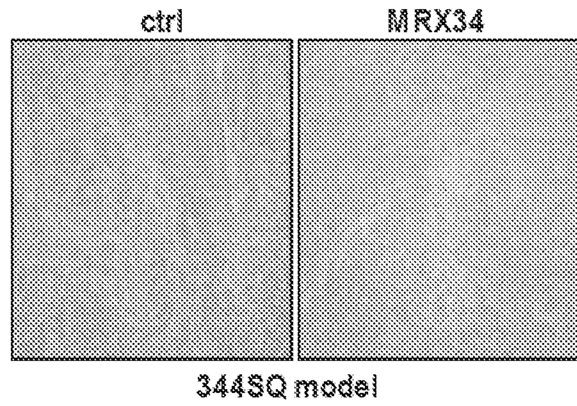


FIG. 4F

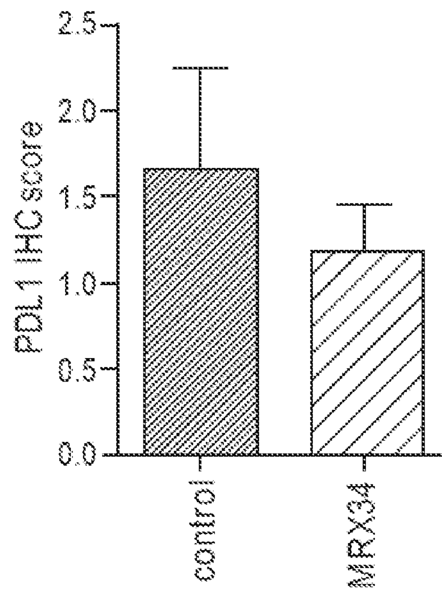


FIG. 4G

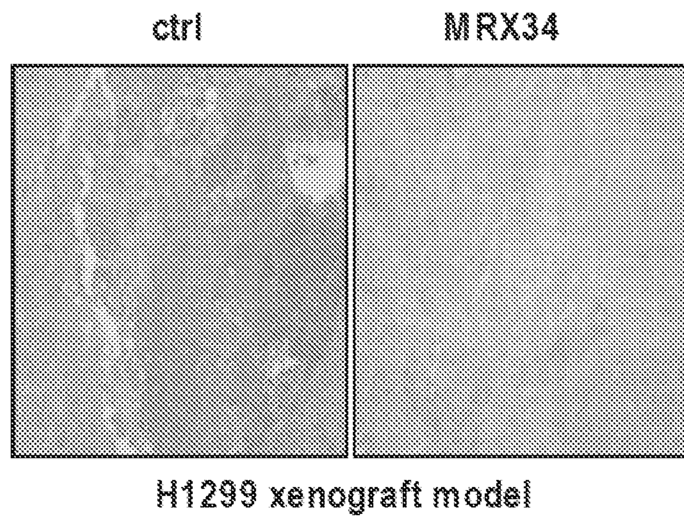


FIG. 4H

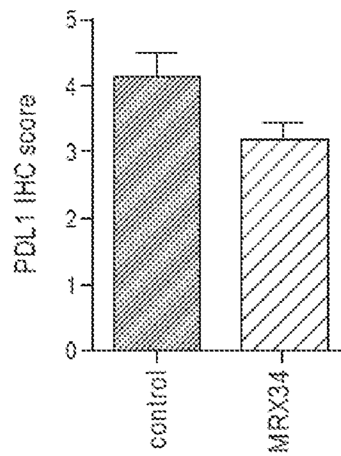


FIG. 5A

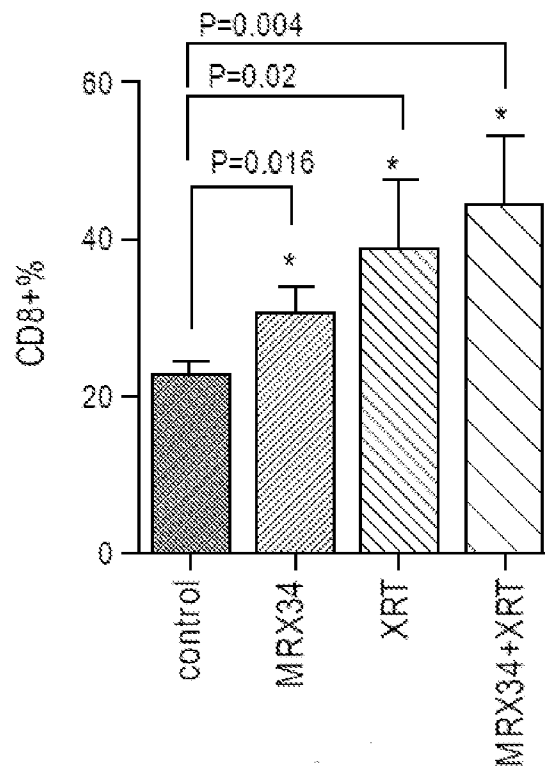


FIG. 5B

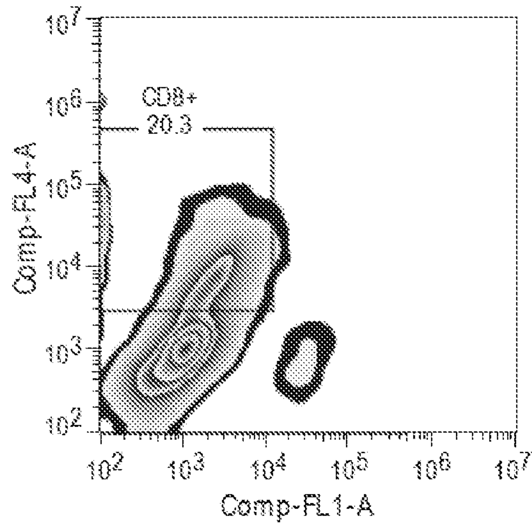


FIG. 5C

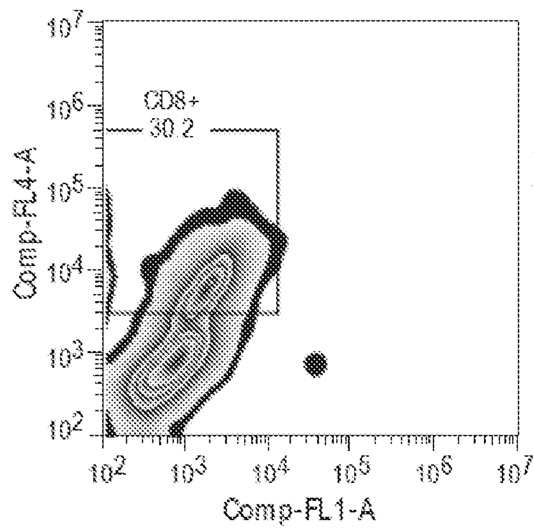


FIG. 5D

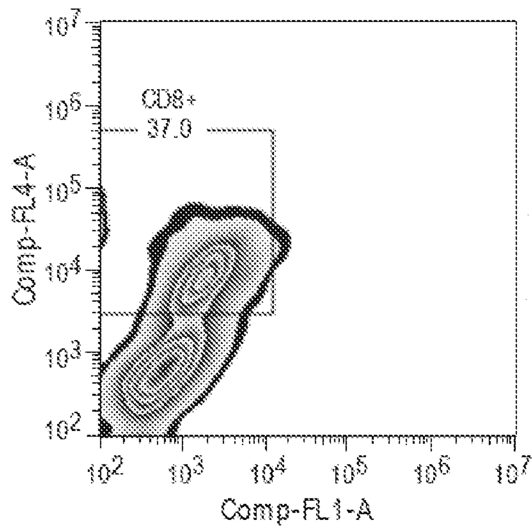


FIG. 5E

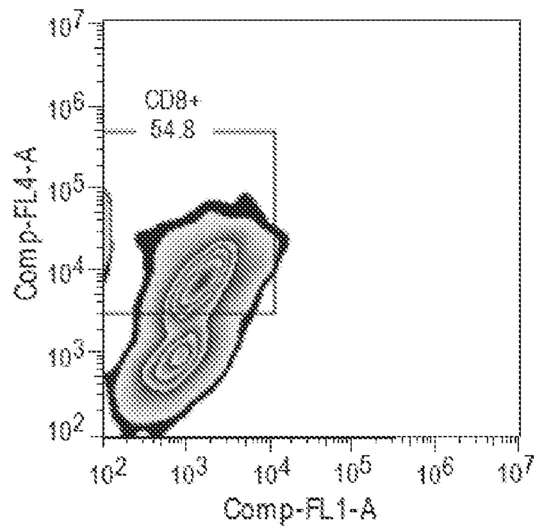


FIG. 5F

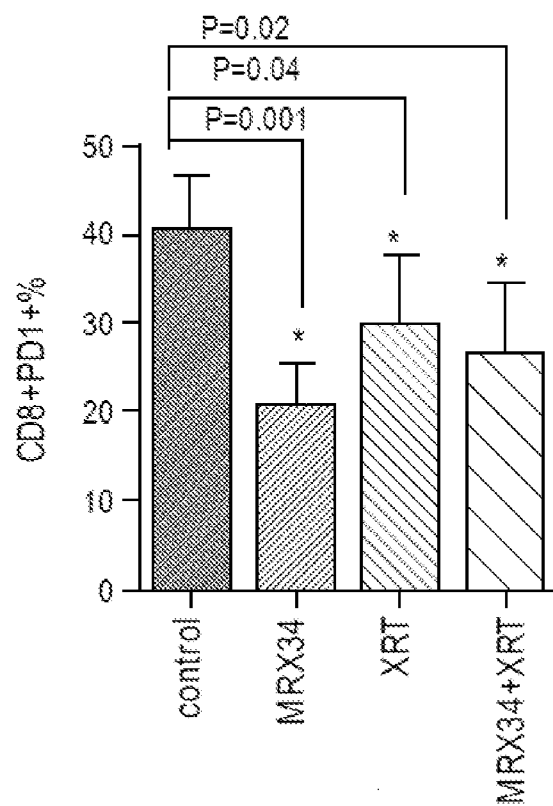


FIG. 5G

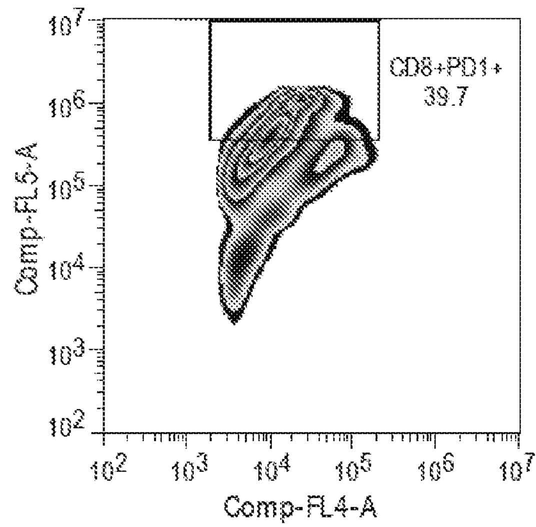


FIG. 5H

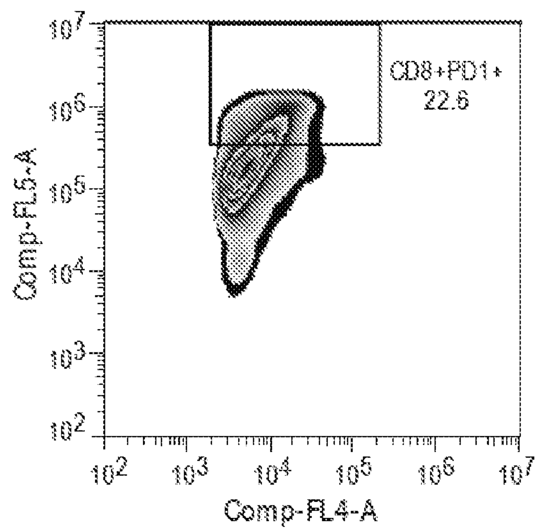


FIG. 5I

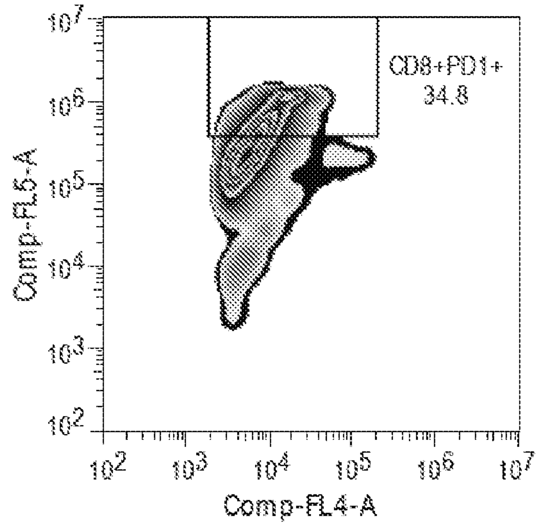


FIG. 5J

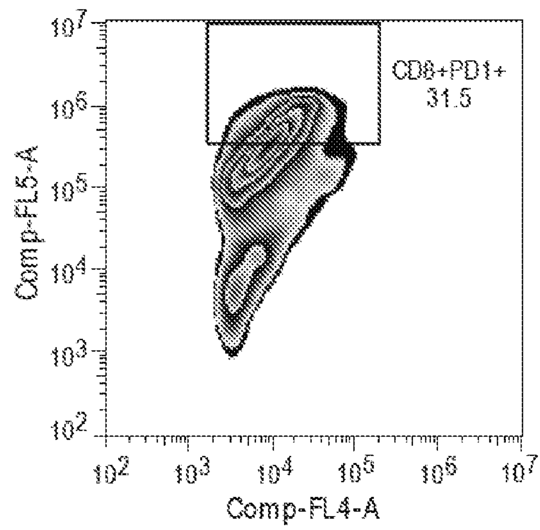


FIG. 5K

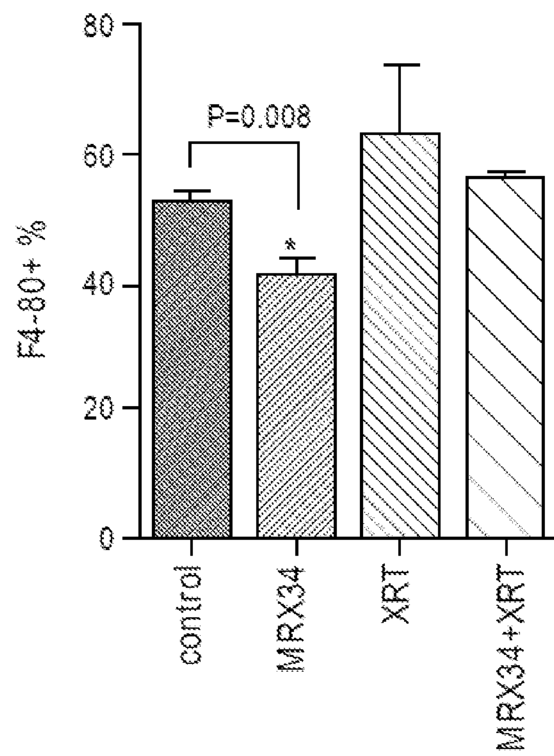


FIG. 5L

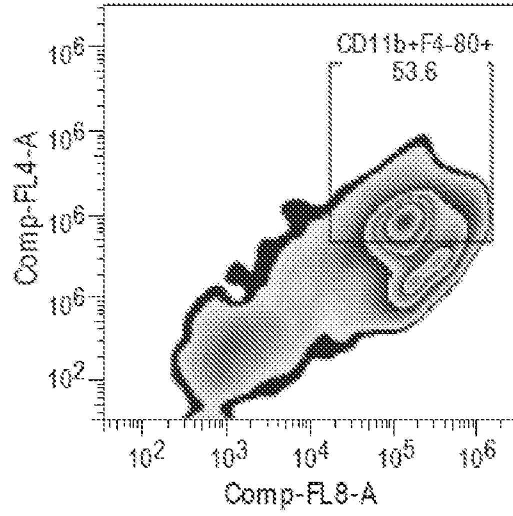


FIG. 5M

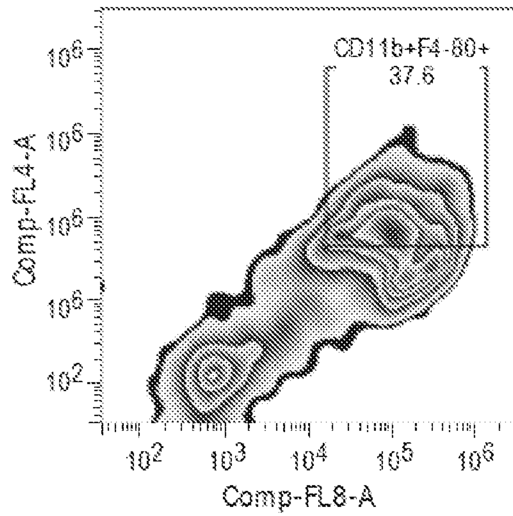


FIG. 5N

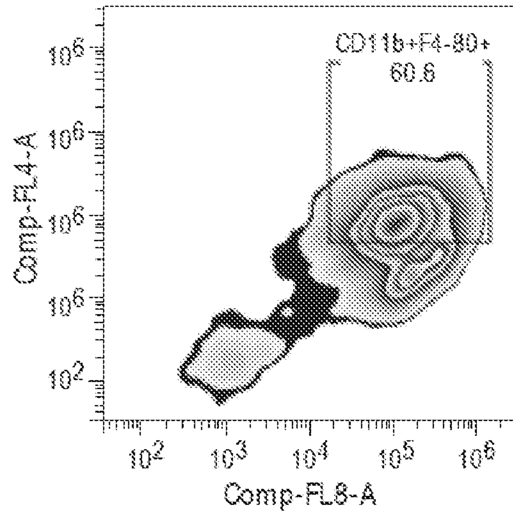


FIG. 5O

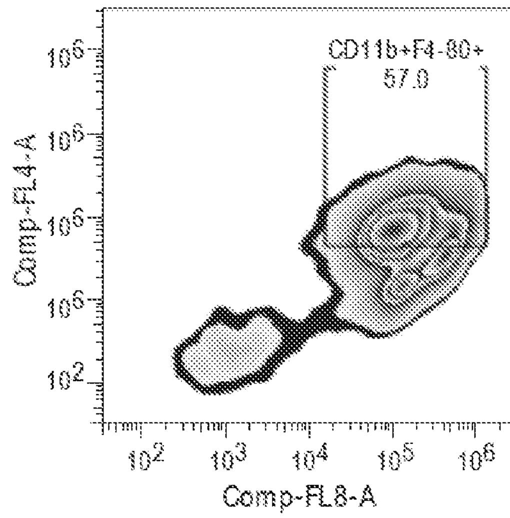


FIG. 5P

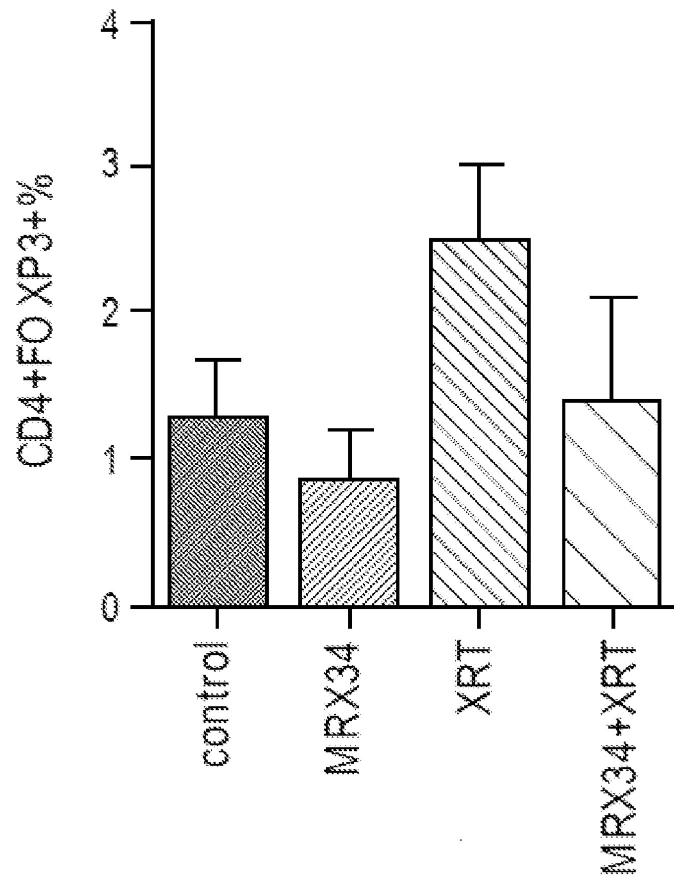


FIG. 5Q

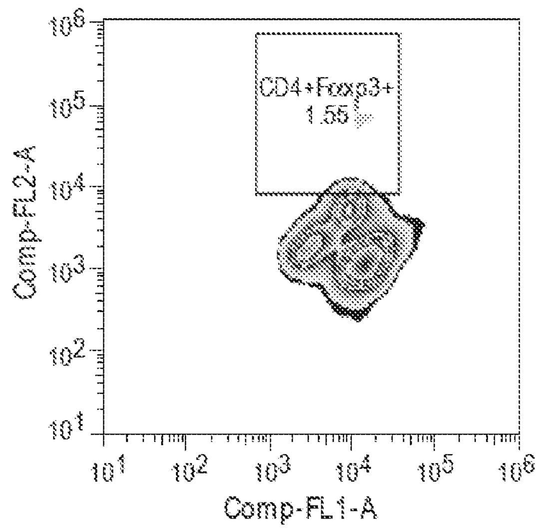


FIG. 5R

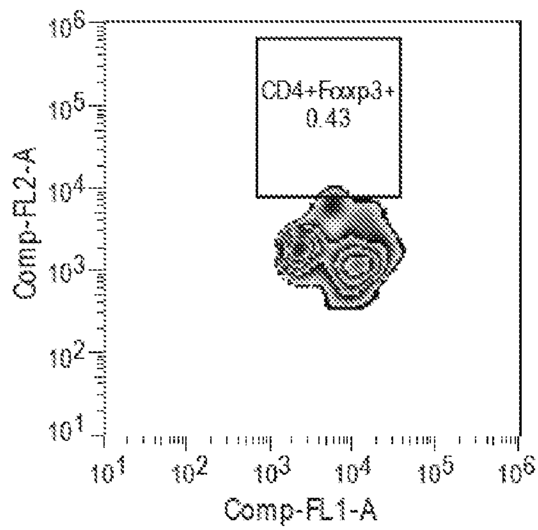


FIG. 5S

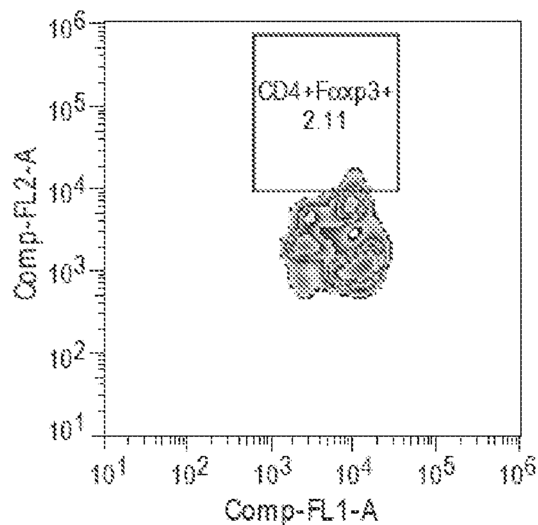


FIG. 5T

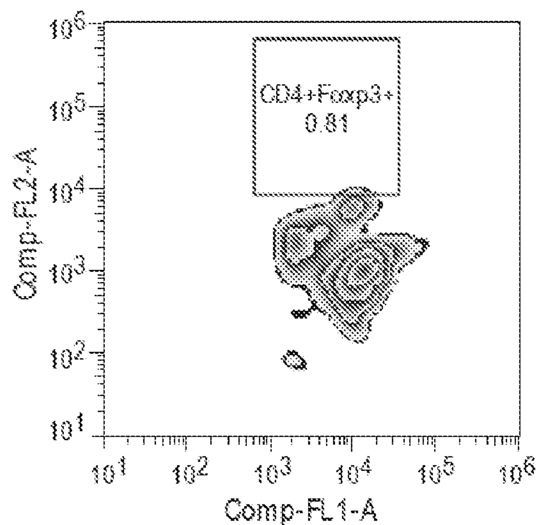


FIG. 5U

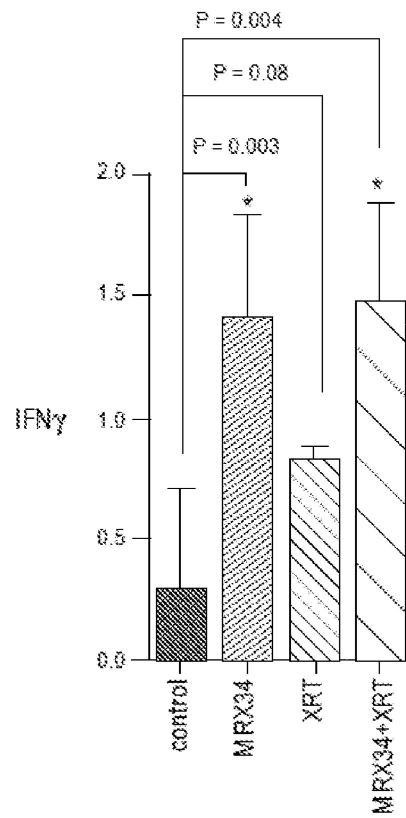


FIG. 5V

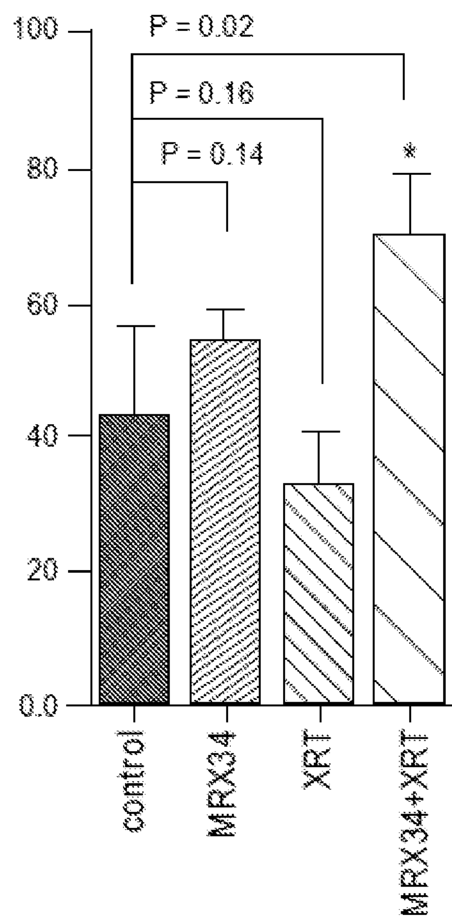
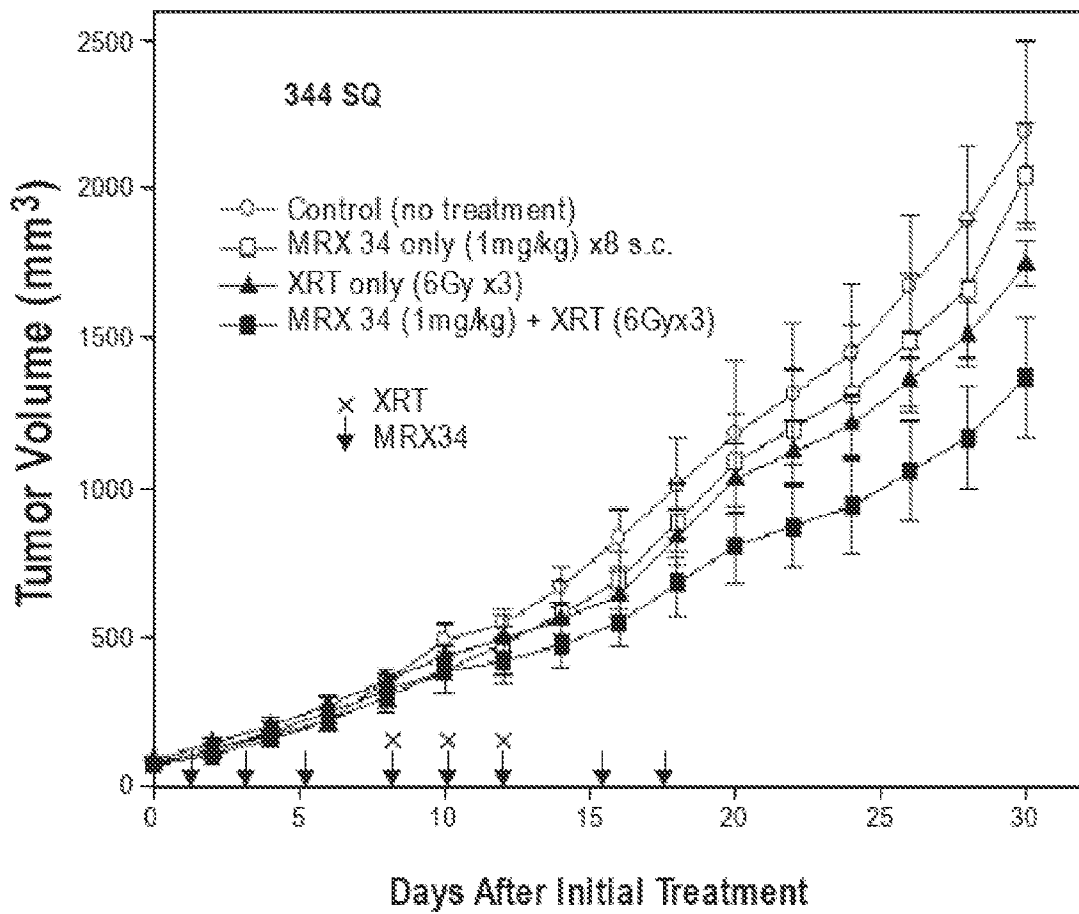


FIG. 5W



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/25410

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/7088, A61K 39/00, A61K 39/395, A61P 35/00, A61P 31/12, A61P 31/04, A61P 31/00 (2016.01)
CPC - A61K 39/3955, A61K 39/0011, C07K 14/4748, A61K 39/3955
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 31/7088, A61K 39/00, A61K 39/395, A61P 35/00, A61P 31/12, A61P 31/04, A61P 31/00 (2016.01)
CPC - A61K 39/3955, A61K 39/0011, C07K 14/4748, A61K 39/3955

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 39/39558, A61K 39/395, C07K 14/71
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar
Search terms: miR-34 OR miR34 OR miRNA-34 OR microRNA-34, PD-1, PD-L1, programmed death, CD274, B7-H1, p53, cancer, tumor, malignancy, GGCAGUG, infection, virus, viral, bacteria, HIV, hepatitis, rhinovirus, herpes, respiratory syncytial virus, RSV, Helicob

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2014/0309278 A1 (MIRNA THERAPEUTICS INC.) 16 October 2014 (16.10.2014) para [0012], [0021], [0041], [0044], [0051], [0055], [0056], [0061], [0073], [0075], [0079], [0086], [0093], [0123]; Table 1; SEQ ID NOs: 1, 2, 168, 169, 170, 171, 172, 173, 174	72-81 --------- 1-71
Y	LIN et al., Programmed Death-Ligand 1 Expression Predicts Tyrosine Kinase Inhibitor Response and Better Prognosis in a Cohort of Patients With Epidermal Growth Factor Receptor Mutation-Positive Lung Adenocarcinoma. Clin Lung Cancer. published online 19 February 2015, Vol 16, No 5, page e25-35 (pp 1-12). Especially p 10, col 1, para 3; p 2, col 2, para 1; p 2, col 2, para 2	1-12, 13-14, 15-37, 38-39, 40-51
Y	US 2011/0271358 A1 (FREEMAN et al.) 03 November 2011 (03.11.2011) para [0024], [0044], [0221]	19, 44, 52-61, 63-71
Y	US 2009/0175827 A1 (BYROM et al.) 09 July 2009 (09.07.2009) para [0047]	13-14, 38-39
Y	OKADA et al., A positive feedback between p53 and miR-34 miRNAs mediates tumor suppression. Genes Dev. 1 March 2014, Vol 28, No 5, pp 438-450. Especially abstract	17, 42, 62

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
01 July 2016

Date of mailing of the international search report
28 JUL 2016

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