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(54) Title: TARGETING KRAS INDUCED IMMUNE CHECKPOINT EXPRESSION

(57) Abstract: The interaction of PD-L1 expressed on the surface of tumor cells triggers PD-1 signaling in T cells which contributes to resistance of cancers to emerging immune checkpoint therapies. The present disclosure demonstrates that KRAS-induced RAF/MEK/ERK/FRA signaling is required for PD-L1 gene expression in tumor cells. Disclosed herein are compositions for the treatment of cancer comprising modulators of KRAS signaling combined with an immunotherapeutic agent that restores the sensitivity of resistant tumor cells to the immunotherapeutic agent. For example, a combination of a KRAS-specific asymmetric interfering RNAs (aiRNAs) and an immune checkpoint inhibitor is shown to enhance tumor cell-specific T cell cytotoxicity. Methods are also described for increasing the therapeutic efficacy of existing anticancer treatments through the co-administration of a modulator of KRAS signaling.

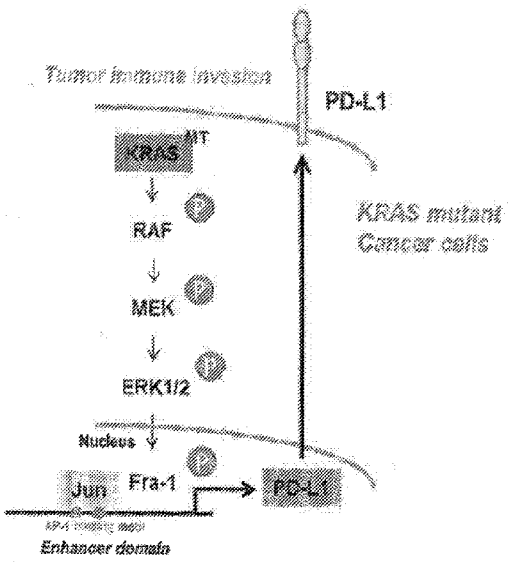

FIG. 8B

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TARGETING KRAS INDUCED IMMUNE CHECKPOINT EXPRESSION

Together with natural killer cells and dendritic cells (DCs), cytotoxic T lymphocytes (CTLs) can orchestrate potent anti-tumor immune responses involving both adaptive and innate effector mechanisms. Nonetheless, tumor cells are often able to evade this immune surveillance by commandeering immune checkpoint inhibitory pathways that are hardwired into the immune system to retain self-tolerance and modulate the duration and amplitude of physiological immune responses in order to minimize potential collateral tissue damage.

The PD-1 immune checkpoint pathway is one such example of an immune checkpoint that has emerged as a critical mediator of immunosuppression in the local tumor microenvironment. The inhibitory co-receptor Programmed Death 1 (PD-1; also known as CD279), a member of the extended CD28/CTLA-4 family of T cell regulators, is expressed on immune cells, such as T, B and NK cells, whereas its ligand, the Programmed Cell Death Ligand 1 (PD-L1, also known as CD274 or B7-H1) is a cell surface glycoprotein expressed on the surface of tumor cells of solid tumors as well as on human tumor associated antigen presenting cells (APCs), e.g., dendritic cells and macrophages. The interaction of PD-L1 ligand on tumor cells with the PD-1 receptor on immune cells delivers an inhibitory signal to T lymphocytes that ultimately leads to T cell anergy and immune evasion.

The development of immune checkpoint inhibitors that prevent the activation of the PD-L1/PD-1 immune checkpoint pathway has resulted in unprecedented and prolonged disease control in about 20-30% of cancer patients with melanoma, non-small cell lung cancer, renal cancer, or head/neck cancer (reviewed by Lipson *et al.*, Semin. Oncol. (2015) 42(4):587-600; Zou *et al.*, Sci. Transl. Med. (2016) vol. 8, issue 328, pp. 328rv4). However, it remains unclear why the remaining 70-80% of cancer patients fail to respond to anti-PD-1 or anti-PD-L1 antibodies or why most patients with colorectal cancer, pancreatic cancer and other non-responsive tumor types are resistant to immune checkpoint inhibitors. Hence, there is a need in the art for treatment modalities targeting cancer cells that are resistant or have acquired resistance to immune checkpoint therapies.

The present disclosure is based on the discovery that aberrant KRAS signaling is at least in part responsible for the activation of PD-L1 gene expression in tumor cells and for the subsequent suppression of tumor cell-specific T cell toxicity. The present disclosure provides compositions and methods that can prevent tumors from evading immune surveillance through

the aberrant activation of the PD-L1/PD-1 immune checkpoint pathway in T cells. In certain embodiments, inhibition of aberrant KRAS signaling in tumor cells sensitizes tumor cells to immune checkpoint inhibitors. In certain embodiments, the disclosure further provides methods for enhancing the therapeutic efficacy of existing anticancer treatment using KRAS signaling modulators.

In one aspect, a composition comprising an effective amount of a modulator of KRAS signaling is disclosed, wherein the modulator of KRAS signaling is effective at enhancing the sensitivity of a tumor cell to tumor cell-specific T cell cytotoxicity. In certain embodiments, the tumor cell is in a subject. In certain embodiments, the modulator of KRAS signaling enhances the efficacy of a therapeutic agent at treating a KRAS associated disease, e.g. cancer.

In certain embodiments, the modulator of KRAS signaling can be, for example, an inhibitor of aberrant KRAS signaling. In certain embodiments, the aberrant KRAS signaling is induced by a modified KRAS, e.g., an oncogenic KRAS, expressed in tumor cells. In certain embodiments, the aberrant KRAS signaling comprises the KRAS induced activation of at least one member of the RAS/RAF/MEK/ERK/FRA-1 signal transduction pathway. In certain embodiments, the aberrant KRAS signaling can result in the KRAS induced activation of PD-L1 gene expression in tumor cells. In certain embodiments, the aberrant KRAS signaling can be induced by an effector of KRAS signaling, e.g. by a KRAS GEF.

In a second aspect, a composition is disclosed that comprises a combination of an effective amount of a modulator of KRAS signaling, and an effective amount of a therapeutic agent, wherein the modulator of KRAS signaling is effective at enhancing the sensitivity of a tumor cell to tumor cell-specific T cell cytotoxicity. In certain embodiments, the modulator of KRAS signaling can be an inhibitor of KRAS signaling. In certain embodiments, the modulator of KRAS signaling can enhance the efficacy of the therapeutic agent at treating a KRAS associated disease, for example, cancer. In certain embodiments, the tumor cell is in a subject.

In a third aspect, a composition is disclosed that comprises a combination of an effective amount of an modulator of oncogenic KRAS signaling, and an effective amount of a therapeutic agent, wherein the modulator of oncogenic KRAS signaling is effective at enhancing the sensitivity of a tumor cell to tumor cell-specific T cell cytotoxicity. In certain embodiments, the modulator of oncogenic KRAS signaling can be an inhibitor of oncogenic KRAS signaling. In certain embodiments, the modulator of oncogenic KRAS signaling can

enhance the efficacy of the therapeutic agent at treating an oncogenic KRAS associated disease. In certain embodiments, the tumor cell is in a subject.

In certain embodiments, the therapeutic agent can be, for example, an anticancer therapeutic agent. In certain embodiments, the anticancer therapeutic agent can be, for example, an immunotherapeutic agent, such as an antigen-binding protein, or fragment thereof, e.g. an antibody that targets a cell surface antigen or extracellular growth factor. In certain embodiments, the anticancer therapeutic agent can be, for example, a small molecule inhibitor of a target protein required for the maintenance or progression of a cancer. In certain embodiments, the small molecule inhibitor can be a small molecule proteasome inhibitor, a small molecule tyrosine kinase inhibitor, a small molecule cyclin-dependent kinase inhibitor, a small molecule inhibitor of a transcription factor or a small molecule inhibitor of an immune checkpoint molecule. In certain embodiments, the anticancer therapeutic agent can be, for example, an RNA interfering agent that silences the expression of a target gene required for the maintenance or progression of a cancer. In certain embodiments, the therapeutic agent can be, for example, an epigenetic inhibitor, e.g. an HDAC inhibitor. In certain embodiments, the anticancer therapeutic agent can be, for example, an anti-estrogen or an anti-androgen therapeutic agent. In certain embodiments, the anticancer therapeutic agent can be, for example, a chemotherapeutic agent and/or radiotherapy. In certain embodiments, the anticancer therapeutic agent can be, for example, a cancer vaccine. In certain embodiments, the anticancer therapeutic agent can be, for example, an RNA interfering agent of an immune checkpoint molecule, e.g. PD-L1.

In a fourth aspect, a composition is disclosed that comprises a combination of an effective amount of a modulator of KRAS signaling, and an effective amount of an immune checkpoint inhibitor, wherein the modulator of KRAS signaling is effective at enhancing the sensitivity of a tumor cell to the immune checkpoint inhibitor. In certain embodiments, the tumor cells can be resistant or have acquired resistance to the immune checkpoint inhibitor. In certain embodiments, the modulator of KRAS signaling sensitizes a tumor cell to tumor cell-specific T cell cytotoxicity. In certain embodiments, the modulator of KRAS signaling enhances the efficacy of the immune checkpoint inhibitor at treating a KRAS associated disease, e.g., cancer. In certain embodiments, the tumor cell is in a subject.

In a fifth aspect, a method for enhancing an immune response against a tumor is disclosed comprising administering an effective amount of a modulator of KRAS signaling and

an effective amount of an immune checkpoint inhibitor to the subject with the cancer, wherein the administration of the modulator of KRAS signaling and the immune checkpoint inhibitor is effective at enhancing the sensitivity of the tumor cells to the immune checkpoint inhibitor. In certain embodiments, the tumor cell is in a subject. In certain embodiments, the modulator of KRAS signaling can act in synergy with the immune checkpoint inhibitor to enhance an immune response against a tumor.

In certain embodiments, the immune checkpoint inhibitor is effective at blocking the interaction of programmed cell death protein 1 (PD-1) receptor with programmed cell death 1 ligand 1 (PD-L1). In certain embodiments, the immune checkpoint inhibitor can be, for example, ipilimumab, tremelimumab, atezolizumab, nivolumab, pembrolizumab, JS001, REGN2810, SHR-1210, MEDI0680, PDR001, BGB-A317, TSR-042, PF-06801591, Ningbo Cancer Hosp. anti-PD-1 CAR, Medimmune anti-PD-1, Isis anti-PD-1, UCB anti-PD-1 or 948.g1, Dana-Farber anti-PD-1, STI-1110, Suzhou Stainwei Biotech anti-PD-1, Haixi pembrolizumab biosimilar, Livzon anti-PD-1, MabQuest anti-PD-1, Singapore ASTR anti-PD-1, Sutro anti-PD-1, Rinat anti-PD-1, Biocad anti-PD-1, Enumeral anti-PD-1 or ENUM 388D4, Kadmon anti-PD-1, BMS-936559, avelumab and/or durvalumab or any combination thereof.

In certain embodiments, the immune checkpoint inhibitor is effective at inhibiting an endogenous immune checkpoint protein or fragment thereof chosen from, for example, PD-1, PD-L1, PD-L2, CD28, CD80, CD86, CTLA4, B7RP1, ICOS, B7RPI, B7- H3, B7-H4, BTLA, HVEM, KIR, TCR, LAG3, CD 137, CD137L, OX40, OX40L, CD27, CD70, CD40, CD40L, TIM3, GAL9, ADORA, CD276, VTCN1, IDOI, KIR3DL1, HAVCR2, VISTA, and/or CD244 or any combination thereof.

In certain embodiments, the tumor cells express a modified KRAS, e.g. an oncogenic KRAS. In certain embodiments, the expressed oncogenic KRAS comprises a mutation of at least one amino acid residue of the amino acid sequence of SEQ ID No.:980. In certain embodiments, the mutation can be an activating mutation of KRAS. In certain embodiments, the oncogenic KRAS comprises an activating mutation of amino acid residues G12, G13, S17, P34 and/or Q61 of SEQ ID No.: 980. In certain embodiments, the oncogenic KRAS comprises a mutation chosen from G12C, G12S, G12R, G12F, G12L, G12N, G12A, G12D, G12V, G13C, G13S, G13D, G13V, G13P, S17G, P34S, Q61K, Q61L, Q61R, and/or Q61H.

In certain embodiments, the modulator of KRAS signaling inhibits aberrant KRAS signaling. In certain embodiments, the aberrant KRAS signaling comprises signaling by an

oncogenic KRAS expressed in the tumor cells. In certain embodiments, the aberrant KRAS signaling comprises the KRAS induced activation of at least one effector of the RAS/ RAF/ MEK/ ERK/ FRA-1 signal transduction pathway in tumor cells. In certain embodiments, the aberrant KRAS signaling comprises the KRAS induced activation of PD-L1 gene expression in tumor cells. In certain embodiments, the modulator of KRAS signaling is ineffective at reducing KRAS induced signaling activity in the absence of oncogenic KRAS gene expression.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the level of KRAS mRNA in the tumor cells by at least about 10%, by at least about 20%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about 60%, by at least about 70%, by at least about 80%, by at least about 90% by at least about 95% or by at least about 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the level of KRAS mRNA in the tumor cells by at least 95%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the level of KRAS mRNA in the tumor cells by at least 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the level of KRAS mRNA in the tumor cells from about 10% to about 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the level of KRAS induced PD-L1 gene expression in the tumor cells by at least about 10%, by at least about 20%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about 60%, by at least about 70%, or by at least about 75% by at least about 80%, by at least about 85%, by at least about 90%, by at least about 95% or by at least about 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the level of KRAS induced PD-L1 gene expression in the tumor cells by at least about 80%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the level of KRAS induced PD-L1 gene expression in the tumor cells from about 10% to about 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the level of FRA-1 gene expression in the tumor cells by at least about 10%, by at least about 20%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about

60%, by at least about 70%, or by at least about 75% by at least about 80%, by at least about 85%, by at least about 90%, by at least about 95% or by at least about 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the level of FRA-1 gene expression in the tumor cells by at least about 80%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the level of FRA-1 gene expression in the tumor cells from about 10% to about 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the KRAS induced activation of at least one effector molecule of the RAS/ RAF/ MEK/ ERK/ FRA-1 signal transduction pathway in tumor cells.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the KRAS induced activation of at least one effector molecule of the RAS/ RAF/ MEK/ ERK/ FRA-1 signal transduction pathway in tumor by at least about 10%, by at least about 20%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about 60%, by at least about 70%, or by at least about 75% by at least about 80%, by at least about 85%, by at least about 90%, by at least about 95% or by at least about 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the KRAS induced activation of at least one effector molecule of the RAS/ RAF/ MEK/ ERK/ FRA-1 signal transduction pathway in tumor from about 10% to about 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the KRAS-induced phosphorylation of RAF, MEK or ERK in tumor cells.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the KRAS-induced phosphorylation of RAF, MEK or ERK in tumor cells by at least about 10%, by at least about 20%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about 60%, by at least about 70%, or by at least about 75% by at least about 80%, by at least about 85%, by at least about 90%, by at least about 95% or by at least about 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the KRAS-induced phosphorylation of RAF, MEK or ERK in tumor cells from about 10% to about 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting the level of both KRAS and PD-L1 gene expression in tumor cells by at least about 10%, by at

least about 20%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about 60%, by at least about 70%, or by at least about 75% by at least about 80%, by at least about 85%, by at least about 90%, by at least about 95% or by at least about 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting both KRAS and PD-L1 gene expression in tumor cells from about 10% to about 99%.

In certain embodiments, the modulator of KRAS signaling can be effective at inhibiting KRAS, RAF, MEK, ERK, FRA-1 and PD-L1 signaling activity in tumor cells from about 10% to about 99%.

In certain embodiments, the modulator of KRAS signaling comprises an RNA interfering agent. In certain embodiments, the RNA interfering agent targets the expression of one or more effectors of the RAS/ RAF/ MEK/ ERK/ FRA-1 signal transduction pathway.

In certain embodiments, the modulator of KRAS signaling comprises an inhibitor of GTP bound KRAS activity. In certain embodiments, the modulator of KRAS signaling comprises an inhibitor of a KRAS GEF. In certain embodiments, the modulator of KRAS signaling comprises an activator of KRAS bound GTP hydrolysis, e.g. KRAS GAP activity. In certain embodiments, the modulator of KRAS signaling comprises an inhibitor of oncogenic KRAS. In certain embodiments, the modulator of KRAS signaling comprises a KRAS-specific RNA interfering agent, e.g. a KRAS-specific asymmetric interfering RNA (referred to herein as KRAS aiRNA or aiKRAS). In certain embodiments, the modulator of KRAS signaling comprises an oncogenic KRAS-specific RNA interfering agent, e.g. an oncogenic KRAS-specific asymmetric interfering RNA. In certain embodiments, the modulator of KRAS signaling comprises an RNA interfering agent that targets both wild type and oncogenic KRAS, e.g. a KRAS-specific asymmetric interfering RNA.

In certain embodiments, the tumor can be, for example, a tumor caused by pancreatic ductal adenocarcinoma (PDAC), colorectal cancer, or non-small-cell lung cancer (NSCLC).

In certain embodiments, the cancer can be a metastatic cancer, a cancer that is refractory to chemotherapy, a cancer that is refractory to radiotherapy and/or a cancer that has relapsed. In certain embodiments, the cancer can be resistant to an immunotherapeutic agent, e.g. an immune checkpoint inhibitor.

In a sixth aspect, the disclosure provides a modulator of oncogenic KRAS signaling.

In certain embodiments, the oncogenic KRAS signaling comprises aberrant KRAS signaling.

In certain embodiments, the modulator of KRAS signaling is effective at inhibiting oncogenic KRAS signaling.

In certain embodiments, the modulator of KRAS signaling is ineffective at reducing KRAS induced signaling activity in the absence of oncogenic KRAS gene expression.

In certain embodiments, the modulator of KRAS signaling comprises an RNA interfering agent, for example, an asymmetric interfering RNA (aiRNA).

In certain embodiments, the asymmetric interfering RNA comprises a sense strand sequence that is at least 50% identical to a sequence chosen from SEQ ID NOs: 320-637.

In certain embodiments, the asymmetric interfering RNA comprises a sense strand sequence chosen from SEQ ID NOs: 320-637.

In certain embodiments, the asymmetric interfering RNA comprises an antisense strand sequence that is at least 50% identical to a sequence chosen from SEQ ID NOs: 638-955.

In certain embodiments, the asymmetric interfering RNA comprises an antisense strand sequence chosen from SEQ ID NOs: 638-955.

In a seventh aspect, the disclosure provides a composition comprising an effective amount of a modulator of oncogenic KRAS signaling.

In an eighth aspect, the disclosure provides a method for changing the efficacy or/and safety of a therapeutic agent comprising administering an effective amount of a modulator of KRAS signaling. In certain embodiments, the modulator of KRAS signaling can act in synergy with the therapeutic agent to enhance the efficacy and/or safety of the therapeutic agent at treating cancer.

In a ninth aspect, the disclosure provides a method for changing the efficacy or/and safety of a therapeutic agent comprising administering an effective amount of an asymmetric interfering RNA (aiRNA). In certain embodiments, the asymmetric interfering RNA (aiRNA) can act in synergy with the therapeutic agent to enhance the efficacy and/or safety of the therapeutic agent at treating cancer.

In a tenth aspect, the disclosure provides a method for changing the efficacy or/and safety of a therapeutic agent comprising administering an effective amount of an asymmetric

interfering RNA (aiRNA) comprising a sense strand sequence chosen from SEQ ID NOs: 320-637.

In an eleventh aspect, the disclosure provides a method for changing the efficacy or/and safety of a therapeutic agent comprising administering an effective amount of an asymmetric interfering RNA (aiRNA) comprising an antisense strand sequence chosen from SEQ ID NOs: 638-955.

In certain embodiments, the efficacy of the therapeutic agent is enhanced.

In certain embodiments, the safety of the therapeutic agent is enhanced.

In certain embodiments, the therapeutic agent is an immune checkpoint inhibitor.

In certain embodiments, the therapeutic agent is chosen, for example, from ipilimumab, tremelimumab, atezolizumab, nivolumab, pembrolizumab, JS001, REGN2810, SHR-1210, MEDI0680, PDR001, BGB-A317, TSR-042, PF-06801591, Ningbo Cancer Hosp. anti-PD-1 CAR, Medimmune anti-PD-1, Isis anti-PD-1, UCB anti-PD-1 or 948.g1, Dana-Farber anti-PD-1, STI-1110, Suzhou Stainwei Biotech anti-PD-1, Haixi pembrolizumab biosimilar, Livzon anti-PD-1, MabQuest anti-PD-1, Singapore ASTR anti-PD-1, Sutro anti-PD-1, Rinat anti-PD-1, Biocad anti-PD-1, Enumeral anti-PD-1 or ENUM 388D4, Kadmon anti-PD-1, BMS-936559, avelumab and/or durvalumab or any combination thereof.

In certain embodiments, the therapeutic agent can be effective at inhibiting an endogenous immune checkpoint protein or fragment thereof chosen from, for example, PD-1, PD-L1, PD-L2, CD28, CD80, CD86, CTLA4, B7RP1, ICOS, B7RPI, B7- H3, B7-H4, BTLA, HVEM, KIR, TCR, LAG3, CD 137, CD137L, OX40, OX40L, CD27, CD70, CD40, CD40L, TIM3, GAL9, ADORA, CD276, VTCN1, IDOI, KIR3DL1, HAVCR2, VISTA, and/or CD244 or any combination thereof.

Other features and advantages of the present disclosure are apparent from the additional descriptions provided herein including the different examples. Based on the present disclosure the skilled artisan may identify and employ other components and methodologies useful for practicing the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A shows the nucleotide sequences within the KRAS gene that are targeted as well as the sequences of the sense and antisense strands of the scrambled aiControl and exemplary

interfering RNAs aiKRAS#1, aiKRAS#2, aiKRAS#3, aiKRAS#4, aiPD-L1, aiFra-1#1, and aiFra-1#2.

FIG.1B depicts an exemplary nucleotide sequence of the human KRAS proto-oncogene, transcript variant B mRNA (NCBI Reference Sequence: NM_004985.4) along with the encoded amino acid sequence of KRAS B (SEQ ID NO.: 981). The locations of the most common activating missense mutations within the KRAS amino acid sequence are highlighted in grey. Nucleotide sequences targeted by the exemplary interfering RNAs aiRNAs#1-4 within SEQ ID NO.: 980 are in bold and underlined.

FIG. 2 shows an exemplary embodiment of aiKRAS silencing of KRAS and PD-L1 expression in $KRAS^{MT}$ cells and $KRAS^{WT}$ cells. **FIG. 2A and FIG. 2B** show an exemplary embodiment of MDA-MB-231 cells treated with aiKRAS#1, aiKRAS#2, aiKRAS#3 and aiKRAS#4 (at 1nM and 0.1nM). PD-L1 expression and KRAS silencing were confirmed by Western Blot (FIG. 2A) and quantitative real-time PCR (FIG. 2B). Data with mean and error bars represent mean of standard error (SEM). FIG. 2C shows an exemplary embodiment of KRAS silencing of PD-L1 expression in MDA-MB-231 cells at 0, 8, 24, 32 and 48 hours post-transfection. FIG. 2D shows an exemplary embodiment of aiKRAS silencing of KRAS and PD-L1 expression in KRAS mutant cells (H358, H460 and H2009) and KRAS wild-type cells (RKO, TCCSUP) transfected with aiKRAS#1 (at 1nM and 0.1nM). The expression of KRAS and PD-L1 was confirmed by Western Blot and quantitative real-time qPCR (data not shown). FIG. 2E shows an exemplary embodiment of the down-regulation of PD-L1 expression resulting from aiRNA mediated silencing of KRAS gene expression in various $KRAS^{MT}$ cell lines.

Fig. 3 shows an exemplary embodiment of aiKRAS silencing of ERK phosphorylation in KRAS mutant ($KRAS^{MT}$) cells. $KRAS^{MT}$ MDA-MB-231 cells and $KRAS^{WT}$ RKO cells were transfected with aiKRAS#1 at 1nM for 48 hours. FIG. 3A shows an exemplary embodiment of $KRAS^{MT}$ MDA-MB-231 and $KRAS^{WT}$ RKO cell lysates applied to a phosphokinase array (R&D systems, USA). FIG. 3B shows an exemplary embodiment of phosphorylated ERK1/2 being inhibited only in $KRAS^{MT}$ MDA-MB-231.

FIG. 4 shows an exemplary embodiment of the effect of aiKRAS silencing on RAS/MEK1/2/ERK1/2 signaling pathway in $KRAS^{MT}$ and $KRAS^{WT}$ cells. $KRAS^{MT}$ MDA-MB-231 cells and $KRAS^{WT}$ RKO cells were treated with aiKRAS#1 at 1nM. After a 48h transfection period, the cell lysate was applied to a Western Blot to confirm the amount of total

or phosphorylated MEK and ERK. FIG. 4A shows an exemplary embodiment of KRAS silencing inhibiting MEK/ERK pathway in $KRAS^{MT}$ MDA-MB-231 cells but not in $KRAS^{WT}$ RKO cells. FIG. 4B shows an exemplary embodiment of inhibition of PD-L1 gene expression and ERK1/2 phosphorylation in MDA-MB-231 cells treated with the MEK inhibitor, U0126. PD-L1 expression and total and phosphorylated ERK were analyzed by Western Blot. FIGs. 4C and 4D show an exemplary embodiment of the failure of KRAS silencing to inhibit STAT3 signaling and nuclear localization of RELA/RELB in $KRAS^{MT}$ MDA-MB-231 cells treated with aiKRAS#1 at 1nM. After a 48h transfection period, the amount of nuclear localization of NF-KB (RELA and RELB) (FIG. 4C) or the amount of whole and phosphorylated STAT3 (FIG. 4D) was determined by Western Blot.

FIG. 5 shows an exemplary embodiment of KRAS silencing inhibiting the phosphorylation, the accumulation of FRA-1 protein as well as the transcriptional activity of *FRA-1* gene. KRAS mutant MDA-MB-231, H358, and H460 cells were treated with aiKRAS#1 at 1nM for 48h. FIG. 5A shows an exemplary embodiment of a Western blot of the cell lysate and the detection of total or phosphorylated FRA-1. FIG. 5B shows an exemplary embodiment of KRAS mutant MDA-MB-231 cells and KRAS wild type RKO cells treated with either control aiRNA or aiKRAS#1. Nuclear extracts (5μg/well) were then incubated in 96-well plates coated with an immobilized oligonucleotide containing the TRE (TPA response element) sequence that is required for AP-1 binding. Competition with TRE oligonucleotides was performed to confirm DNA binding specificity. FIG. 5B shows an exemplary embodiment of an analysis of the complexes formed in the RAS mutant or KRAS wild type cells by ELISA assay in the presence of FRA-1-specific antibodies. FIG. 5C shows an exemplary embodiment of a ChIP-qPCR analysis of the PD-L1 enhancer. ChIP was conducted in aiControl or aiKRAS treated KRAS mutant MDA-MB-231 cells and KRAS wild type RKO cells. The predicted PD-L1 enhancer sequence was enriched in immunoprecipitated chromatin using an anti-FRA-1 antibody and an anti-cJUN antibody in KRAS mutant cells (Fig. 5C Left) but not in KRAS wild type cells (Fig.5D Right), or in chromatin incubated with the negative control IP (normal rabbit IgG). Data with mean and error bars represent mean of standard error (SEM).

FIG. 6 shows an exemplary embodiment of the effects of FRA-1 on PD-L1 expression in KRAS mutant cells. KRAS mutant MDA-MB-231 and H460 cells were treated with control aiRNA, aiKRAS, and two different FRA-1 aiRNAs (aiFra-1 #1, and aiFra-1 #2). PD-L1 and FRA-1 expression were confirmed using Western Blot (Fig. 6A) and qPCR (Fig. 6B). Data with mean and error bars represent mean of standard error (SEM).

FIG. 7 shows an exemplary embodiment of cytotoxic T cell activity against KRAS mutant cancer cells after aiKRAS silencing of PD-L1 expression. FIG. 7A shows an exemplary embodiment of cytotoxic T lymphocyte (CTL) activity against KRAS mutant MDA-MB-231 cells transfected with aiKRAS. CMV-specific CTLs were expanded to culture human PBMC with HLA-A*02:01 CMV pp65 peptide. aiRNA-transfected Luc-MDA-MB-231 cells were incubated with or without CMV peptide. Peptide loaded or non-loaded MDA-MB-231 cells were plated into 96-well plates (2000 cells/well). CMV-specific CD8+ T cells were subsequently added to 96-well plates with Effector : Target (E/T) ratio of 50:1 and incubated for 24 hours. Live Luc-MDA-MB-231 cells were measured for intracellular luciferase activity with D-Luciferin K+ salt. The percent lysis was then calculated as: (Luminescence of CMV peptide pulsed Luc-MDA-MB-231/ Luminescence of CMV peptide un-pulsed Luc-MDA-MB-231) x 100. Anti-PD-L1 antibody (10μg/mL) was used as a positive control. Data with mean and error bars represent mean of standard error (SEM). Data were subjected to one-way ANOVA with Dunnett's multiple comparison of means test. Statistical significance is displayed as p-value ** $p<0.01$ and *** $p<0.001$. FIG. 7B shows an exemplary embodiment of PD-L1 cell surface expression on MDA-MB-231 cells after aiKRAS and aiPD-L1 transfection. An exemplary embodiment of the calculated geometric mean of fluorescence intensity (MFI) measured by flow cytometry is depicted at the bottom.

FIG. 8A shows an exemplary embodiment of some of the canonical KRAS signaling pathways. In certain embodiments, growth factor binding to cell-surface receptors results in activated receptor complexes, which contain adaptors such as SHC (SH2-containing protein), GRB2 (growth-factor-receptor bound protein 2) and GAB (GRB2-associated binding) proteins. These proteins can recruit SHP2 and SOS1, a guanine nucleotide exchange factors (RAS GEF) protein that can increase RAS–guanosine triphosphate (RAS–GTP) levels by catalyzing nucleotide exchange on RAS. In contrast, the GTPase-activating protein (GAP) neurofibromin (NF1) can bind to RAS–GTP and accelerate the conversion of RAS–GTP to inactive RAS–GDP (guanosine diphosphate), which can terminate signaling. The BRAF–mitogen-activated and extracellular-signal regulated kinase-kinase (MEK)–extracellular signal-regulated kinase (ERK) cascade can determine key cellular processes including cell proliferation. RAS can also activate the phosphatidylinositol 3-kinase (PI3K) – 3-phosphoinositide-dependent protein kinase 1 (PDK1)–AKT pathway that can determine cellular survival. RALGDS, RALGDS-like gene (RGL), RGL2 and TIAM1 can be exchange factors of RAL and RAC, respectively. Among the effectors of RAL is phospholipase D (PLD) an enzyme that can regulate vesicle

trafficking. RAC can regulate actin dynamics and, therefore, the cytoskeleton. RAS can also bind and activate the enzyme phospholipase Cepsilon (PLCepsilon), the hydrolytic products of which can regulate calcium signaling and the protein kinase C (PKC) family. FIG. 8B shows a proposed signaling mechanism along the KRAS/MEK/ERK/Fra-1/PD-L1 axis. In KRAS mutant cancer cells, mutant KRAS can activate MEK/ERK kinase. Stabilized phosphorylated FRA-1 can bind to the PD-L1 enhancer region where it can activate and maintain PD-L1 gene expression in tumor cells.

The methods and techniques of the present application are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present disclosure unless otherwise indicated. See, e.g., M.R. Green and J. Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012), Ausubel *et al.*, Current Protocols, John Wiley & Sons, Inc. (2000-2016), Antibodies: A Laboratory Manual, 2nd edition, edited by Edward A. Greenfield, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2014), and RNA: A Laboratory Manual by Rio *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2011), all of which are incorporated herein by reference.

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

As used herein, the singular terms "a," "an," and "the" include the plural reference unless the context clearly indicates otherwise.

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

than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the disclosure and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

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

When a range of values is listed herein, it is intended to encompass each value and sub-range within that range. For example, "1-5 mg" or "1 to 5 mg" is intended to encompass 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 1-2 mg, 1-3 mg, 1-4 mg, 1-5 mg, 2-3 mg, 2-4 mg, 2-5 mg, 3-4 mg, 3-5 mg, and 4-5 mg.

As used herein, a "polynucleotide" refers to a polymeric chain containing two or more nucleotides. "Polynucleotides" includes primers, oligonucleotides, nucleic acid strands, etc. A polynucleotide may contain standard or non-standard nucleotides. Typically, a polynucleotide

contains a 5' phosphate at one terminus ("5' terminus") and a 3' hydroxyl group at the other terminus ("3' terminus) of the chain. The most 5' nucleotide of a polynucleotide may be referred to herein as the "5'-terminal nucleotide" of the polynucleotide. The most 3' nucleotide of a polynucleotide may be referred to herein as the "3'-terminal nucleotide" of the polynucleotide.

The term "subject" generally refers to an organism to which a compound or pharmaceutical composition described herein can be administered. A subject can be an animal or animal cell, including a mammal or mammalian cell (e.g., a human or human cell). The term also refers to an organism, which includes a cell or a donor or recipient of such cell. In various embodiments, the term "subject" refers to any animal (e.g., a mammal), including, but not limited to, humans, mammals and non-mammals, such as non-human primates, mice, rabbits, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, fish, nematode, and insects, which is to be the recipient of a compound or pharmaceutical composition described herein. Under some circumstances, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

The terms "administer," "administering," or "administration" are used herein in their broadest sense. These terms refer to any method of introducing to a subject a compound or pharmaceutical composition described herein and can include, for example, introducing a compound systemically, locally, or in situ to the subject. Thus, a compound of the present disclosure produced in a subject from a composition (whether or not it includes the compound) is encompassed by these terms. When these terms are used in connection with the term "systemic" or "systemically," they generally refer to *in vivo* systemic absorption or accumulation of the compound or composition in the blood stream and its distribution throughout the entire body. In certain embodiments, the terms "administer," "administering," or "administration" can refer to, for example, delivering one or more recombinant vectors to a tumor cell, wherein the vector expresses an RNA interfering agent as defined herein. In certain embodiments, the tumor cell is in a subject.

The terms "combination," "combinatorial," or "combination treatment," as used herein, mean the administration of at least two different agents (e.g., at least one compound chosen from modulators of KRAS signaling and/or at least one compound chosen from therapeutic agents, and, optionally, one or more additional agents) to treat a disorder, condition, or symptom, e.g., a cancer condition. Such combination treatment may involve the administration of one agent before, during, and/or after the administration of a second agent. The first agent

and the second agent can be administered concurrently, separately, or sequentially in separate pharmaceutical compositions. The first agent and the second agent may be administered by the same or different routes of administration. In certain embodiments, a treatment combination comprises a therapeutically effective amount of at least one compound chosen from modulators of KRAS signaling and a therapeutically effective amount of at least one compound chosen from therapeutic agents, e.g. immune checkpoint inhibitors. In certain embodiments, the immune checkpoint inhibitor can be, for example, an inhibitor of PD-L1.

For example, the at least one compound chosen from modulators of KRAS signaling and at least one compound chosen from therapeutic agents can have different mechanisms of action. In certain embodiments, a combination treatment improves the prophylactic or therapeutic effect of the at least one compound chosen from modulators of KRAS signaling and the at least one compound chosen from therapeutic agents by functioning together to have an additive, synergistic, or enhanced effect. In certain embodiments, a combination treatment of the present disclosure reduces adverse side effects associated with the at least one compound chosen from modulators of KRAS signaling and the at least one compound chosen from therapeutic agents. The administration of the at least one compound chosen from modulators of KRAS signaling and the at least one compound chosen from therapeutic agents may be separated in time by up to several weeks, but more commonly within 48 hours, and most commonly within 24 hours.

As used herein, a "therapeutic agent" that may administered with a modulator of KRAS signaling can be an anticancer therapeutic agent, i.e. an agent that may be administered *in vivo* to treat cancer. In certain embodiments, the anticancer therapeutic agent can be a small molecule, a peptide, a modified peptide, a peptidomimetic, an antibody, an antibody fragment, a recombinant antibody, a recombinant antigen-binding protein, an aptamer, a nucleic acid or RNA interfering agent.

In certain embodiments, the anticancer therapeutic agent can be, for example, a chemotherapeutic agent, i.e. a chemical compound useful in the treatment of cancer. Exemplary classes of chemotherapeutic agents include, but are not limited to, alkylating agents, antimetabolites, kinase inhibitors, mitotic inhibitors, spindle poison plant alkaloids, cytotoxic /antitumor antibiotics, topisomerase inhibitors, photosensitizers, anti-estrogens and selective estrogen receptor modulators (SERMs), anti-progesterones, estrogen receptor down-regulators (ERDs), estrogen receptor antagonists, luteinizing hormone-releasing hormone agonists, anti-

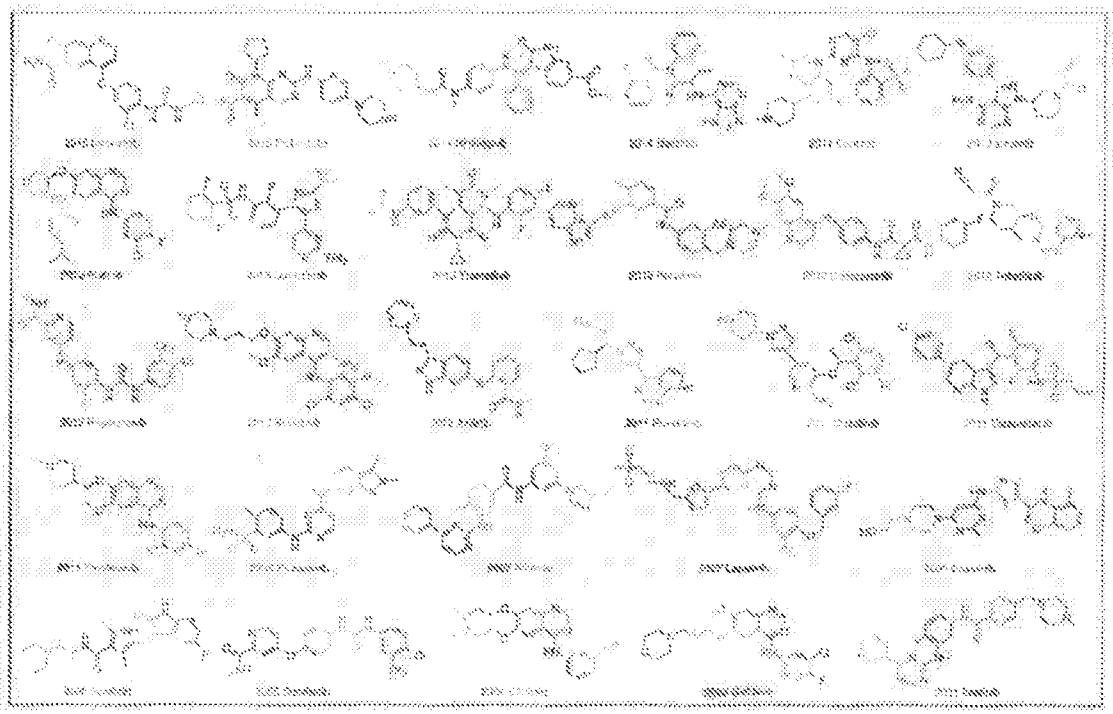
androgens, aromatase inhibitors, EGFR inhibitors, angiogenesis inhibitors, VEGF inhibitors, and inhibitors of the translation and/or transcription of genes implicated in abnormal cell proliferation or tumor growth. Chemotherapeutic agents useful in the treatment methods disclosed herein include cytostatic and/or cytotoxic agents.

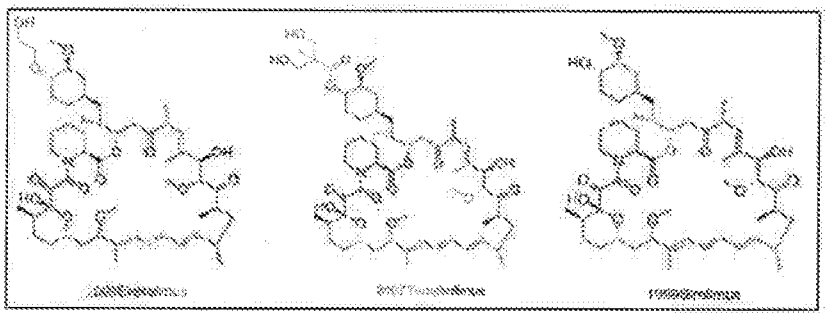
In certain embodiments, the therapeutic agent can be, for example, a biotherapeutic agent, such as an antibody or recombinant antigen-binding protein. In certain embodiments, the antibody or recombinant antigen-bind protein can block ligand / receptor signaling in any biological pathway that supports tumor maintenance and/or growth or suppresses the anti-tumor immune response. In certain embodiments, the therapeutic agent can be, for example, a targeted therapeutic agent, such as a small molecule drug. In certain embodiments, the therapeutic agent can include radiation and/or surgery.

In certain embodiments, the therapeutic agent can be, for example, a small molecule such as a small molecule kinase inhibitor (SMKI). SMKIs that can be combined with a modulator of KRAS signaling are disclosed, for example, in Wu *et al.* Drug Discovery Today (2016) volume 21, issue 1, pp. 5–10, which is incorporated herein by reference.

Exemplary SMKIs are depicted in TABLE 1 below (dates indicate year when FDA approved).

TABLE 1





The terms "effective amount" and "therapeutically effective amount" refer to the amount of a compound or pharmaceutical composition described herein that is capable of invoking, for example, one or more of the following effects: (1) inhibition, to some extent, of cancer or tumor growth, including a decrease or cessation in the progression of cancer; (2) reduction in the number of cancer or tumor cells; (3) reduction in tumor size; (4) inhibition, e.g., a decrease or a cessation, of cancer or tumor cell infiltration into peripheral organs; (5) inhibition, e.g., a decrease or a cessation, of metastasis; (6) enhancement of anti-tumor immune response, which may, but is not required to, result in the regression or rejection of the tumor, or (7) relief, to some extent, of one or more symptoms associated with the cancer or tumor. The therapeutically effective amount can vary depending upon the intended application (*in vitro* or *in vivo*), the subject and disease condition being treated, e.g., the sex, weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art, e.g., a board-certified oncologist. A "therapeutically effective amount" is an amount of a compound where any toxic or detrimental effects resulting from the administration of the compound are outweighed by the therapeutically beneficial effects.

The terms "progress," "progressed," and "progression" as used herein refer to at least one of the following: (1) a response of progressive disease to prior therapy (e.g., chemotherapy and/or immune checkpoint therapy); (2) the appearance of one or more new lesions after treatment with prior therapy (e.g., chemotherapy and/or immune checkpoint therapy); and (3) at least a 5% (e.g., 10%, 20%) increase in the sum of diameters of target lesions, taking as a reference the smallest sum on study.

The term "sensitize" means to alter cancer cells or tumor cells in a way that allows for more effective treatment of the associated cancer with a cancer therapy. In certain embodiments, normal cells are not affected to an extent that causes the normal cells to be unduly injured by the cancer therapy. In certain embodiments, an increased sensitivity or a

reduced sensitivity to a therapeutic treatment can be measured according to a known method in the art for the particular treatment and methods described herein below, including, but not limited to, cell proliferative assays (Tanigawa *et al.* Cancer Res 1982; 42: 2159-2164) or cell death assays (Weisenthal *et al.* Cancer Res 1 84; 94: 161 - 173; Weisenthal *et al.* Cancer Treat Rep 1985; 69: 615-632; Weisenthal *et al.* Drug Resistance in Leukemia and Lymphoma. Langhorne, P A: Harwood Academic Publishers, 1993: 41 -432; Weisenthal L M, Contrib Gynecol Obstet 1994; 19: 82-90). The sensitivity or resistance may also be measured in animals by measuring the tumor size reduction over a period of time, for example, 6 months for humans and 4-6 weeks for mice. A composition or a method sensitizes cancer cells or tumor cells to a therapeutic treatment if the increase in treatment sensitivity or the reduction in resistance is about 25% or more, for example, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90% or more, compared to treatment sensitivity or resistance in the absence of such composition or method. In certain embodiments, the increase in treatment sensitivity or the reduction in resistance is about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 10-fold, about 15-fold, about 20-fold or more compared to treatment sensitivity or resistance in the absence of such composition or method. The determination of sensitivity or resistance to a therapeutic treatment is routine in the art and within the skill of an ordinarily skilled clinician. It is to be understood that any method described herein for enhancing the efficacy of a cancer therapy can be applied to methods for sensitizing hyperproliferative or otherwise cancerous cells (e.g., resistant cells) to the cancer therapy.

The term "synergy," "synergistic," "synergistically," or "enhanced" as used herein refers to an effect of interaction or combination of two or more components to produce a combined effect greater than the sum of their separate effects (or "additive effects"). A synergistic effect may be attained when the compounds are: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g. in separate tablets, pills or capsules, or by different injections in separate syringes. A synergistic anticancer effect denotes an anticancer effect which is greater than the predicted purely additive effects of the individual compounds of the combination administered separately.

Terms such as "treating" or "treatment" or "to treat" as used herein refer to (1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a

diagnosed pathologic condition or disorder or/and (2) prophylactic or preventative measures that prevent or slow the development and/or progression of a targeted pathologic condition or disorder. Thus those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. A subject is successfully "treated" according to the methods of the present disclosure if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including the spread of cancer into soft tissue and bone; inhibition of or an absence of tumor metastasis; inhibition or an absence of tumor growth; relief of one or more symptoms associated with a specific cancer; reduced morbidity and mortality; and improvement in quality of life. In certain embodiments, "treating cancer," "treatment of cancer," or an equivalent thereof, mean preventing recurrence of cancer after surgical removal or other anticancer therapies.

As used herein, a "modulator" refers to a compound or combination of compounds that is capable of modulating KRAS signaling activity, including but not limited to, oncogenic KRAS signaling or otherwise aberrant KRAS signaling activity.

In certain embodiments, a "modulator" can refer to a compound or combination of compounds that is capable of modulating the expression a target gene required for KRAS signaling.

In certain embodiments, a "modulator" can refer to a compound or combination of compounds that are capable of modulating the expression of KRAS.

In certain embodiments, a "modulator" can refer to a compound or combination of compounds that are capable of modulating the expression of one or more of KRAS, RAF, MEK, ERK and FRA-1.

As used herein, "modulating" and its grammatical equivalents refer to either increasing or decreasing (e.g., silencing), in other words, either up-regulating or down-regulating KRAS signaling activity, e.g., by about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, 99% or 100%, compared to KRAS signaling activity in the absence of a modulator.

In certain embodiments, a "modulator" can refer to an inhibitor of a cellular activity, e.g. an inhibitor of oncogenic KRAS signaling.

In certain embodiments, a "modulator" can refer to an activator of a cellular activity, e.g. KRAS GAP induced hydrolysis of GTP bound to KRAS.

As used herein, the terms "inhibiting", "to inhibit" and their grammatical equivalents, when used in the context of a bioactivity, refer to a down-regulation of the bioactivity, which may reduce or eliminate the targeted function, such as the production of a protein or the phosphorylation of a molecule. When used in the context of an organism (including a cell), the terms refer to a down-regulation of a bioactivity of the organism, which may reduce or eliminate a targeted function, such as the production of a protein or the phosphorylation of a molecule. In particular embodiments, inhibition may refer to a reduction, e.g., of about 10%, of about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%, or about 100% of the targeted activity. When used in the context of a disorder or disease, the terms refer to success at preventing the onset of symptoms, alleviating symptoms, or eliminating the disease, condition or disorder.

The term "cancer" in a subject refers to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain morphological features. Often, cancer cells will be in the form of a tumor or mass, but such cells may exist alone within a subject, or may circulate in the blood stream as independent cells, such as leukemic or lymphoma cells.

Examples of cancer as used herein include, but are not limited to lung cancer, pancreatic cancer, bone cancer, skin cancer, head or neck cancer, cutaneous or intraocular melanoma, breast cancer, uterine cancer, ovarian cancer, peritoneal cancer, colon cancer, microsatellite instability-high metastatic colorectal cancer, microsatellite stable metastatic colorectal cancer, colorectal cancer with mismatch-repair deficiency, colorectal cancer without mismatch-repair deficiency, small bowel adenocarcinoma, rectal cancer, colorectal adenocarcinoma, cancer of the anal region, stomach cancer, gastric cancer, gastrointestinal cancer, gastric adenocarcinoma, adrenocorticoid carcinoma, genitourinary cancer, gynecologic cancer, uterine cancer, uterine sarcoma, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the vagina, carcinoma of the vulva, cervical cancer, Hodgkin's Disease, esophageal cancer, gastroesophageal junction cancer, gastroesophageal adenocarcinoma, chondrosarcoma, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, Ewing's sarcoma, cancer of the urethra, cancer of the penis, prostate cancer, bladder

cancer, testicular cancer, cancer of the ureter, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, kidney cancer, renal cell carcinoma, endometrial cancer, chronic or acute leukemia, multiple myeloma, lymphocytic lymphomas, neoplasms of the central nervous system (CNS), spinal axis tumors, brain cancer, brain stem glioma, glioblastoma multiforme, astrocytomas, schwannomas, ependymomas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenomas, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers. Some of the exemplified cancers are included in general terms and are included in this term. For example, urological cancer, a general term, includes bladder cancer, prostate cancer, kidney cancer, testicular cancer, and the like; and hepatobiliary cancer, another general term, includes liver cancers (itself a general term that includes hepatocellular carcinoma or cholangiocarcinoma), gallbladder cancer, biliary cancer, or pancreatic cancer. Both urological cancer and hepatobiliary cancer are contemplated by the present disclosure and included in the term "cancer."

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

In certain embodiments, the cancer is breast cancer. In certain embodiments, the cancer is colorectal adenocarcinoma. In certain embodiments, the cancer is small bowel adenocarcinoma. In certain embodiments, the cancer is hepatocellular carcinoma. In certain embodiments, the cancer is head and neck cancer. In certain embodiments, the cancer is renal cell carcinoma. In certain embodiments, the cancer is ovarian cancer. In certain embodiments, the cancer is prostate cancer. In certain embodiments, the cancer is lung cancer. In certain embodiments, the cancer is uterine sarcoma. In certain embodiments, the cancer is esophageal cancer. In certain embodiments, the cancer is pancreatic cancer. In certain embodiments, the cancer is a gastric cancer. In certain embodiments, the cancer is endometrial cancer. In certain embodiments, the cancer is cholangiocarcinoma. In certain embodiments, each of the cancers is unresectable, advanced, refractory, recurrent, or metastatic. In certain embodiments, the

cancer is resistant or has acquired resistance to an anticancer therapeutic agent, e.g. treatment with an immune checkpoint inhibitor.

In certain embodiments, the efficacy of a compound or a combination of compounds is tested in a xenograft cancer model in which cells isolated from a solid tumor are injected into a host animal, e.g. an immunocompromised host, to establish solid tumors. In certain embodiments, the cells isolated from a solid tumor comprise cancer stem cells. The host animal can be a model organism such as nematode, fruit fly, zebrafish; preferably a laboratory mammal such as a mouse (nude mouse, SCID mouse, or NOD/SCID mouse, Beige /SCID Mouse), rat, rabbit, or primate. The severely immunodeficient NOD-SCID mice may be chosen as recipients to maximize the participation of injected cells.

Solely to provide certain background and not to limit the scope of this disclosure, a *KRAS* gene (also called C-K-RAS; CFC2; K-RAS2A; K-RAS2B; K-RAS4A; K-RAS4B; KI-RAS; KRAS1; KRAS2; NS; NS3; RASK2) encodes the human cellular homolog of a transforming gene isolated from the Kirsten rat sarcoma virus. KRAS is a member of the mammalian RAS gene family that encode a group of closely related 21 kDa GDP/GTP-binding proteins that can act as intracellular signal transducers. Alternative splicing of the Human *KRAS* pre-mRNA generates transcript variants "A" and "B" encoding two isoforms that differ in the C-terminal region. Human transcript variant "B" having the exemplary nucleotide sequence of SEQ ID NO.: 980 (see FIG. 1B; Accession No.: NM_004985), the prevalent KRAS isoform, comprises five exons but lacks exon 4a which the longer transcript variant "A" includes.

As used herein, KRAS protein refers to a polypeptide having at least about 40%, e.g., about 80%, identity to the amino acid sequence provided at Genbank Accession No. AAB41942 or ABY87538.

In certain embodiments, the KRAS protein can refer to a polypeptide comprising at least 10 contiguous amino acids of the amino acid sequence of SEQ ID NO.: 981.

In certain embodiments, the KRAS protein refers to the KRAS isoform B having the exemplary amino acid sequence of SEQ ID NO.: 981.

1 MTEYKLVVVG AGGVGKSALT IQLIQNHFVD EYDPTIEDSY RKQVVIDGET CLLDILDTAG

61 QEEYSAMRDQ YMRTGEGFLC VFAINNTKSF EDIHHYREQI KRVKDSEDVP MVLVGNKCDL

121 PSRTVDTKQA QDLARSYGIP FIETSAKTRQ GVEDAFYTLV REIRKHKEKM SKDGKKKKKK

181 SKTKCVIM (SEQ ID NO.: 981)

Solely to provide certain background and not to limit the scope of this disclosure, the N-terminal portion (residues 1–165) of KRAS comprises a highly conserved G domain which is also found in H-RAS and N-RAS isoforms. RAS proteins can diverge substantially at the C-terminal end, which is known as the hypervariable region. This region can contain residues that specify post-translational protein modifications that are essential for targeting RAS proteins to the cytosolic leaflet of cellular membranes. All RAS proteins are farnesylated at a terminal CAAX motif, in which C is cysteine, A is usually an aliphatic amino acid, and X is any amino acid. KRAS4A is additionally modified by one or two palmitic acids just upstream of the CAAX motif. The addition of the hydrophobic farnesyl moiety is complemented by the hydrophobic palmitates (the so-called "second signal") to firmly anchor KRAS4A to the membrane. By contrast, KRAS4B, the predominant splice variant, contains an alternative second signal that is composed of a polybasic stretch of lysine residues. In this case, membrane anchoring is mediated by the electropositive lysines that form ionic bonds to the predominantly electronegative lipid head groups of the inner leaflet of the plasma membrane.

In certain embodiments, the term "KRAS" encompasses wild type KRAS4A and/or KRAS4B. In certain embodiments, the term "KRAS" encompasses both wild type and modified forms of KRAS4A and/or KRAS4B.

In certain embodiments, modified forms of KRAS include, but are not limited to, KRAS proteins having one or more activating mutations, for example, missense mutations at positions G12, G13 and/or Q61. In certain embodiments, KRAS encompasses KRAS proteins having one or more alterations in the post-translational modifications of KRAS, including, but not limited to, acetylation, methylation, lipidation, palmitoylation, prenylation, and S-nitrosylation.

Tethered to the inner leaflet of the plasma membrane, KRAS can act as a binary molecular switch at the apex of a signaling hub where it can control the transmission of signals from cell surface receptors to intracellular effectors by cycling between a GDP-bound inactive

and a transient GTP-bound active state. In its active GTP-bound form, KRAS can activate downstream effectors that control cellular processes in the cytoplasm (actin organization, endocytosis) or modify the activity of nuclear transcription factors that regulate gene expression important for cell cycle progression, differentiation, or survival.

The KRAS molecular switch can function by responding to upstream signals by activating a class of proteins known as guanine nucleotide exchange factors (RAS GEFs) that can stimulate the dissociation of GDP from the RAS protein. For example, SOS1, a RAS GEF in the MAPK/ERK pathway, can be recruited by the adaptor protein GRB2 in response to epidermal growth factor receptor (EGFR) activation. The binding of SOS1 (Son of Sevenless 1) to GBR2 can localize it to the plasma membrane, where it can activate membrane bound RAS. Activation of KRAS by release of GDP then can allow RAS to bind free GTP which can trigger a conformational change in the protein's structure that can facilitate the transient transduction of extracellular signals from the active GTP-bound RAS to downstream signaling pathways. The inactive GDP bound KRAS can then be restored by GTPase acting proteins (GAPs), like p120GAP, that enhance GTP hydrolysis by the otherwise slow intrinsic GTPase activity of RAS proteins.

Once activated, RAS-GTP can preferentially bind to and activate downstream RAS-binding-domain (RBD) or RAS-association (RA)-domain-containing effectors. It can be estimated that there are at least 11 distinct RAS effector families, each of which can activate a distinct protein signaling cascade. Exemplary downstream effector pathways that respond to the KRAS activation are depicted in FIG. 8A and summarized below.

One exemplary downstream effector of activated GTP-bound KRAS is the RAS-RAF-MAP-MEK-ERK kinase cascade which can be an essential, shared element of mitogenic signaling involving tyrosine kinase receptors that leads to a wide range of cellular responses, including growth, differentiation, inflammation, and apoptosis. GTP-bound RAS can recruit RAF serine/threonine kinases (A-RAF, B-RAF and C-RAF-1). The interaction of activated GTP-bound KRAS with RAF can initiate the RAF → MEK → ERK kinase cascade. Activated RAF, a MAPK (Mitogen-activated protein) kinase-kinase, can phosphorylate the MAPK kinase proteins MEK1 and MEK2 (also known as MAP2K1 and MAP2K2), which in turn can phosphorylate the proline-directed kinases ERK1 and 2 (extracellular signal-regulated kinase; also known as MAPK3 and MAPK1). Activated ERK1/2 (p42/44 MAP kinase) can then phosphorylate many substrates, including kinases that are important for control of translation

(e.g., p90RSK) and transcription factors that control genes involved in cell cycling (e.g., ELK1, FOS, MYC, FRA-1)

Another exemplary downstream effector of activated GTP-bound KRAS is the RAS-phosphoinositide 3-kinase (PI3K)-AKT signaling pathway involved in cell survival and proliferation. PI3Ks are heterodimeric lipid kinases composed of a regulatory subunit (p85) and a catalytic subunit (p110). GTP-bound RAS bound to the p110 (α, β, γ, and δ) catalytic subunits of class I PI3K can trigger the synthesis of the secondary messenger, phophatidylinositol-3,4,5-triphosphate (PIP3). PIP3 can then be free to engage the pleckstrin homology (PH) domain of AKT/PKB (Protein kinase B), thereby stimulating its Ser/Thr kinase activity and the phosphorylation of a host of other proteins involved in cell growth, cell cycle entry, and cell survival. AKT-mediated phosphorylation can inhibit some proteins that promote programmed cell death (BAD, FoxO), and stimulates others (MDM2) that promote cell survival.

Many additional RAS effectors have now been identified. These include RAIN, an endomembrane receptor for RAS, NORE1, a pro-apoptotic tumor suppressor, and AF-6, a mediator of membrane-cytoskeleton interactions. RAS can also engage in cross- talk with other GTPase signaling pathways involved in regulating actin reorganization and/ or endocytic trafficking. This can occur through interactions of RAS with RAL guanine nucleotide dissociation stimulator for the RAS-like (RAL) small GTPases, RALA and RALB, a GEF that facilitates GDP/GTP exchange with RAS (RALGDS). RAS can also interact with GEFS like RIN1, for RAB5, and TIAM1 (tumor invasion and metastasis inducing protein 1), for RAC. In addition, accumulating evidence indicates signaling specificity may also be dictated by differential localization of KRAS isoforms in discrete plasma membrane microdomains or distinct intracellular membrane compartments (e.g., endosomes, Golgi), where the activated GTPase may encounter a unique sets of effectors.

KRAS can be essential for mammalian embryonic development. KRAS-deficient mice can die of anemia and defective fetal liver erythropoiesis after only about 12–14 days of gestation. Germline mutations that affect components of the RAS–RAF–MEK-ERK pathway can cause several developmental disorders, including Noonan Syndrome (NS3), Costello Syndrome and Cardio-Facio-Cutaneous (CFC2) syndrome. The developmental disorders associated with RAS pathway mutations may share phenotypic features that include facial

abnormalities, heart defects, impaired growth and development, and, in some instances, a predisposition to specific cancers.

KRAS is also a proto-oncogene. A proto-oncogene can become oncogenic by increased KRAS expression or the acquisition of an "activating" mutation, i.e. a mutation that leads to constitutive aberrant activation of KRAS signaling. Indeed, activating somatic KRAS mutations have been detected in ~30% of all human cancers. Amongst those cancers, KRAS can be found in a predominantly mutated form in pancreatic ductal adeno-carcinoma (71%), colorectal cancer (35%), non-small cell lung adenocarcinoma (19%), and endometrial cancer (17%) (see TABLE 2 below; collated from the Catalogue of Somatic Mutations in Cancer (COSMIC) database). Activating KRAS gene point mutations can also be present in other cancers, including but not limited to, biliary tract malignancies, endometrial cancer, cervical cancer, bladder cancer, liver cancer, myeloid leukemia and breast cancer.

TABLE 2

Primary Tissue	*KRAS* (%)	*HRAS* (%)	*NRAS* (%)	Total (%)
Pancreas	71	0	<1	71
Colon	35	1	6	42
Small intestine	35	0	<1	35
Biliary tract	26	0	2	28
Endometrium	17	<1	5	22
Lung	19	<1	1	20
Skin (melanoma)	1	1	18	20
Cervix	8	9	2	18
Urinary tract	5	10	1	16

Mutant KRAS, as used herein, refers to somatic or germline KRAS4A and/or KRAS4B mutations including, but not limited to, point mutations, nonsense substitutions, missense substitutions, synonymous substitutions, in frame insertions, frameshift insertions and/or deletions. KRAS missense gain-of-function activating mutations can be found predominantly at one of three mutational hotspots: G12 (89%), G13 (9%), and Q61 (1%). In certain embodiments, these mutations disrupt intrinsic as well as GAP-mediated GTP hydrolysis. In certain embodiments, the disruption results in an accumulation of constitutively active GTP-bound RAS in cancer cells.

In certain embodiments, a mutant KRAS can refer to aberrant post-translational modification of KRAS. In certain embodiments, the aberrant post-translational modification of KRAS includes, but not limited to, phosphorylation, glycosylation, ubiquitination,

nitrosylation, methylation, acetylation, lipidation (C-terminal glycosyl phosphatidylinositol (GPI) anchor, N-terminal myristoylation, S-myristoylation, S-prenylation) and/or proteolysis.

In certain embodiments, a mutant KRAS can refer to aberrant splicing of KRAS mRNAs.

TABLE 3 below provides an exemplary list of KRAS4B mutations and their reported association with human cancers and developmental disorders. Oncogenic KRAS mutations associated with cancer include, without limitation, $KRAS^{G12D}$, $KRAS^{G12V}$, $KRAS^{G13D}$, $KRAS^{G12C}$, $KRAS^{Q61R}$, $KRAS^{Q61L}$, $KRAS^{Q61K}$, $KRAS^{G12R}$, and $KRAS^{G12C}$. The skilled artisan will understand that a *KRAS* gene comprising a different KRAS mutation than one of those above and/or combinations of the above and/or other KRAS mutations that lead to constitutive activation of KRAS signaling, is also an oncogenic KRAS encompassed by the present disclosure. A comprehensive list of KRAS mutations present in human cancer is available online from UniProt Consortium, EMBL.

TABLE 3

EXEMPLARY KRAS MUTATIONS[1]

PHENOTYPE	MUTATION
Lung Cancer, Somatic	KRAS, GLY12CYS
Lung Cancer, Squamous Cell, Somatic Bladder Cancer, Somatic, Included	KRAS, GLY12ARG
Breast Adenocarcinoma, Somatic Juvenile Myelomonocytic Leukemia, Somatic, Included Ras-Associated Autoimmune Leukoproliferative Disorder, Somatic, Included	KRAS, GLY13ASP
Bladder Cancer, Transitional Cell, Somatic	KRAS, ALA59THR
Pancreatic Carcinoma, Somatic Gastric Cancer, Somatic, Included Epidermal Nevus, Somatic, Included Nevus Sebaceous, Somatic, Included Schimmelpenning-Feuerstein-Mims Syndrome, Somatic Mosaic, Included Juvenile Myelomonocytic Leukemia, Somatic, Included Ras-Associated Autoimmune Leukoproliferative Disorder, Somatic, Included	KRAS, GLY12ASP
Pancreatic Carcinoma, Somatic Nevus Sebaceous, Somatic, Included	KRAS, GLY12VAL
Gastric Cancer, Somatic Juvenile Myelomonocytic Leukemia, Somatic, Included	KRAS, GLY12SER
Leukemia, Acute Myelogenous, Somatic	KRAS, 3-BP INS, GLY11INS

[1] reproduced from OMIM® (Online Mendelian Inheritance in Man®), Johns Hopkins University.

PHENOTYPE	MUTATION
Cardiofaciocutaneous Syndrome 2	KRAS, GLY60ARG
Cardiofaciocutaneous Syndrome 2 Noonan Syndrome 3, Included	KRAS, ASP153VAL
Noonan Syndrome 3	KRAS, THR58ILE
Noonan Syndrome 3	KRAS, VAL14ILE
Cardiofaciocutaneous Syndrome 2	KRAS, PRO34ARG
Noonan Syndrome 3	KRAS, VAL152GLY
Noonan Syndrome 3	KRAS, ASP153VAL
Pilocytic Astrocytoma, Somatic	KRAS, GLY13ARG
Cardiofaciocutaneous Syndrome 2	KRAS, LYS5ASN
Cardiofaciocutaneous Syndrome 2	KRAS, PHE156LEU
Noonan Syndrome 3	KRAS, LYS5GLU
Noonan Syndrome 3	KRAS, GLY60SER
Cardiofaciocutaneous Syndrome 2	KRAS, TYR71HIS
Cardiofaciocutaneous Syndrome 2	KRAS, LYS147GLU
Ras-Associated Autoimmune Leukoproliferative Disorder, Somatic	KRAS, GLY13CYS
Variant Of Unknown Significance	KRAS, LEU19PHE

In certain embodiments, "KRAS signaling" can refer to wild type KRAS4A and/or KRAS4B GTPase activity, GTP/GDP binding activity or any signaling activity induced by GTP-bound KRAS4A and/or KRAS4B, including, but not limited to, the RAS-RAF-MAP-MEK-ERK, the RAS-PI3Ks-AKT and RAS-RalGDS signal transduction pathways as summarized in part above (e.g., see FIG. 8A).

In certain embodiments, "KRAS signaling" encompasses any form of aberrant KRAS signaling. For example, in certain embodiments, aberrant KRAS signaling can occur as a result of signaling by an oncogenic KRAS protein having one or more activating mutations, including, but not limited to, missense mutations at positions G12, G13 and/or Q61.

In certain embodiments, the aberrant KRAS signaling refers to the activity of a hyperactive wild type GTP bound KRAS as a result of changes in GDP–GTP regulation, loss of GAPs or persistent receptor tyrosine kinase-mediated activation of GEFs. Thus, in certain embodiments, aberrant KRAS signaling can be caused by the aberrant activation of effector molecules downstream of KRAS, including, but not limited to, A-RAF, B-RAF, C-RAF, MEK, ERK and/or FRA-1. In certain embodiments, aberrant KRAS signaling, e.g. oncogenic KRAS signaling, can occur in the presence of wild type KRAS. In certain embodiments, aberrant KRAS signaling, e.g. oncogenic KRAS signaling, can occur as a result of the overexpression of KRAS, e.g. via gene amplification.

In certain embodiments, KRAS signaling comprises hyperactive KRAS signaling initiated as a result of the inactivation of a tumor suppressor that, when inactivated, provides an alternative mechanism of activating RAS. In certain embodiments, the hyperactive KRAS signaling results from the inactivation of a tumor suppressor such as a RAS GAP. Exemplary RAS GAPs include, but are not limited to, RASA1, RASA2, RASA3, RASA4, RASAL1, NF1, DAB2IP, RASAL2, RASAL3, SynGAP1, IQGAP1, IQGAP2 and IQGAP3.

The aberrant signaling by mutant or otherwise modified KRAS has been implicated in developmental disorders and virtually all aspects of the malignant phenotype of the cancer cell, including cellular proliferation, transformation, invasion and metastasis. However, despite the importance of oncogenic KRAS in the etiology of human cancers, efforts to develop small molecule drugs targeting, for example, oncogenic KRAS over the past three decades have been largely unsuccessful.

For example, as reported by Stephen *et al.*, Cancer Cell (2014) vol. 25, 3 272–281, efforts to block RAS associated cancers by preventing RAS farnesylation, once thought to be an essential posttranslational modification for RAS activity, were thwarted by the unexpected presence of a backup system (geranylgeranyltransferase) that restored activity of KRAS and NRAS after farnesyltransferase treatment. Likewise, efforts to block one of RAS' major downstream effectors, RAF kinase, ran into the unexpected discovery that, in RAS-transformed cells, RAF inhibitors activate the pathway rather than inhibit it. MAP kinase-kinase (MEK) inhibitors and phosphatidylinositol 3-kinase (PI3K) inhibitors have not yet shown significant clinical activity in RAS associated cancers, for reasons relating to feedback loops and poor therapeutic windows as well as lack of specificity. This failure has led some to dismiss KRAS as an "undruggable" target. Even strategies employing siRNAs to target mutant KRAS remain challenging primarily because of off target silencing of genes unrelated to KRAS or the induction of a robust interferon response.

In one aspect, the present disclosure reports on an approach for specifically inhibiting aberrant KRAS signaling in tumor cells without the known caveats associated with RNA interference.

As shown in Examples 2 and 3, oncogenic KRAS signaling in KRAS associated cancer cell lines can induce aberrant RAF/MEK/ERK/FRA-1 signaling that can stimulate the constitutive high level expression of the immune checkpoint, PD-L1. The interaction of PD-L1 expressed on the surface of tumor cells with PD-1 receptor on T cells can trigger the activation

of the PD-L1/PD-1 immune checkpoint pathway in T cells which can lead to the suppression of tumor cell-specific T cell cytotoxicity. In certain embodiments, the targeted inhibition of KRAS signaling, e.g., by KRAS-specific asymmetric interfering RNAs, can down-regulate PD-L1 gene expression and restore the sensitivity of cancer cells expressing an oncogenic KRAS to killing by antigen-specific cytotoxic T cells (see Example 4). In certain embodiments, the inhibition of oncogenic KRAS signaling in cancer cells can inhibit MEK/ERK-dependent phosphorylation. In certain embodiments, the inhibition of oncogenic KRAS signaling in cancer cells can inhibit the accumulation of FRA-1 protein. In certain embodiments, FRA-1 protein is a transcription factor required for the activation of the AP-1 responsive enhancer within the first intron of the PD-L1 gene. Inhibition of KRAS signaling may therefore provide a novel approach to sensitizing cancer cells resistant to immunotherapies, such as immune checkpoint therapies, as well as improving the efficacy of known anticancer therapeutics.

In certain embodiments, the modulator of KRAS signaling may comprise, for example, an inhibitor that reduces or prevents KRAS-mediated cell signaling in tumor cells. In certain embodiments, the tumor cell expresses an oncogenic KRAS. In certain embodiments, the inhibitor can directly target both wild type and/or oncogenic KRAS by inhibiting the expression of KRAS in tumor cells. In certain embodiments, the inhibitor of KRAS can be, for example, an RNA interfering agent.

An "RNA interfering agent," as used herein, is defined as any agent that can inhibit the expression of a target gene by RNA interference (RNAi).

Solely to provide a background and not to limit the scope of this disclosure, "RNA interference (RNAi)" is an evolutionally conserved process whereby the expression or introduction of an RNA comprising a sequence that is identical to or highly similar to a target gene sequence can result in the sequence-specific degradation or specific post-transcriptional gene silencing (PTGS) of messenger RNA (mRNA) transcribed from that targeted gene, thereby inhibiting target gene expression. In nature, RNAi is initiated by the dsRNA-specific endonuclease, Dicer, a member of RNase III ribonuclease family. Dicer cleaves long, double-stranded RNA (dsRNA), pre-microRNA (miRNA), and short hairpin RNA (shRNA) into short double-stranded RNA fragments called small interfering RNAs (siRNA) of about 20-25 nucleotides in length, usually with a two-base overhang on the 3' end. Dicer catalyzes the first step in the RNA interference pathway and initiates formation of the RNA-induced silencing complex (RISC), whose catalytic component, argonaute, is an endonuclease capable of

degrading messenger RNA (mRNA) whose sequence is complementary to that of the siRNA guide strand.

RNAi can be initiated by introducing nucleic acid molecules, e.g., synthetic siRNAs or RNA interfering agents, having a guide RNA that targets a specific expressed gene sequence. Examples of such RNA interfering agents include, but are not limited to, small non-coding RNAs such as antisense oligonucleotides, shRNAs (e.g. as disclosed in U.S. Patent No. 7,750,144, which is incorporated by reference herein in its entirety for any purpose), siRNAs (e.g. as disclosed in U.S. Patent No. 7,056,704 and 9,260,470, which are incorporated by reference herein in their entireties for any purpose), a microRNA or a mature microRNA molecule or a pre-microRNA molecule or a primary microRNA molecule, or a variant thereof, (e.g. as disclosed in U.S. Patent No. 8,609,831, which is incorporated by reference herein in its entirety for any purpose), gapmers (e.g. as disclosed in U.S. Patent No. 6,107,094, which is incorporated by reference herein in its entirety for any purpose), lncRNA (e.g. as disclosed in the International Publication No. WO2012018881, which is incorporated by reference herein in its entirety for any purpose), a piRNA (piwiRNA) molecule (e.g. as disclosed in the International Publication No. WO2008109142, which is incorporated by reference herein in its entirety for any purpose), a triplex oligonucleotide (e.g. as disclosed in U.S. Patent No. 5,693,773, which is incorporated by reference herein in its entirety for any purpose), or ribozymes (e.g. as disclosed in U.S. Patent No. 5,225,347, which is incorporated by reference herein in its entirety for any purpose).

Exemplary chemical modifications of RNA interfering agents are disclosed in Dar *et al.* siRNAmod: A database of experimentally validated chemically modified siRNAs. Sci. Rep. (2016) 6, 20031.

The present disclosure provides compositions comprising a class of short double stranded RNA interfering agents, called asymmetrical interfering RNAs (aiRNA), that can induce potent gene silencing in mammalian cells. aiRNA is described, for example, in PCT Publications WO 2009/029688 and WO 2009/029690, the contents of which are hereby incorporated by reference in their entireties for any purpose. In one aspect, this class of RNAi-inducers is characterized in the length asymmetry of the two RNA strands. This structural design can not only be functionally potent in effecting gene silencing but offer several advantages over the current state-of-art siRNAs. Among the advantages, aiRNA can have RNA duplex structure of much shorter length than the other siRNA, which should reduce the cost of

synthesis and abrogate/reduce the length-dependent triggering of nonspecific interferon-like responses. In addition, the asymmetry of the aiRNA structure abrogates and/or otherwise reduces the sense-strand mediated off-target effects. aiRNA is therefore, in certain embodiments, more efficacious, more potent, with a more rapid-onset, and more durable at inducing gene silencing than any of the other RNA interfering agents.

In certain embodiments, aiRNAs disclosed herein each comprises a first strand with a length from 18-23 nucleotides (nt) and a second strand with a length from 12-17 nucleotides. In certain embodiments, the second strand is substantially complementary to the first strand. In certain embodiments, the second strand forms a double-stranded region with the first strand. In certain embodiments, the first strand has a 3'-overhang from 1-9 nucleotides. In certain embodiments, the first strand has a 5'-overhang from 0-8 nucleotides. In certain embodiments, the aiRNA is capable of effecting at silencing KRAS signaling in a eukaryotic cell.

In certain embodiments, the first strand is 18, 19, 20, 22, or 23 nucleotides long.

In certain embodiments, the second strand is 12, 13, 14, 15, 16, or 17 nucleotides long.

In certain embodiments, the 3'-overhang is greater than 0 nucleotides in length. In certain embodiments, the first strand comprises a sequence being substantially complementary to a target KRAS mRNA sequence. In certain embodiments, the first strand comprises a sequence being at least 70 percent complementary to a target mRNA sequence.

In certain embodiments, the first strand is at least 1 nt longer than the second strand. In a further embodiment, the first strand is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nt longer than the second strand. In another embodiment, the first strand is 20-100 nt longer than the second strand. In a further embodiment, the first strand is 2-12 nt longer than the second strand. In an even further embodiment, the first strand is 3-10 nt longer than the second strand.

In certain embodiments, the first strand, or the long strand, has a length of 5-100 nt, or preferably 10-30 or 12-30 nt, or more preferably 15-28 nt. In one embodiment, the first strand is 21 nucleotides in length. In some embodiments, the second strand, or the short strand, has a length of 3-30 nt, or preferably 3-29 nt or 10-26 nt, or more preferably 12-26 nt. In some embodiments, the second strand has a length of 15 nucleotides.

In certain embodiments, the double-stranded region has a length of 3-98 base pairs (bp). In a further embodiment, the double-stranded region has a length of 5-28 bp. In an even further

embodiment, the double-stranded region has a length of 10-19 bp. The length of the double-stranded region can be 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 bp.

In certain embodiments, aiRNAs disclosed herein comprise a duplex RNA comprising a first strand, wherein the 5'-terminal and 3'-terminal nucleotides of the first strand are from 16 to 21 nucleotides apart, and a second strand, wherein the 5'-terminal and 3'-terminal nucleotides of the second strand are from 10 to 17 nucleotides apart, wherein the 5'-terminal nucleotide of the second strand is complementary to a nucleotide of the first strand other than its 3'-terminal nucleotide, wherein the 3'-terminal nucleotide of the second strand is complementary to a nucleotide of the first strand.

In certain embodiments,the penultimate nucleotide from the 3'-terminal nucleotide of the first strand is not dT.

In certain embodiments, the duplex RNA is more effective at silencing an expressed nucleotide sequence of a target gene than a corresponding 21-mer siRNA duplex targeting the same expressed nucleotide sequence of the target gene.

In certain embodiments, the second strand is from 1to 9 nucleotides shorter than the first strand. In certain embodiments, the 5'-terminal and 3'-terminal nucleotides of the first strand are 19 nucleotides apart. In certain embodiments, the 5'-terminal and 3'-terminal nucleotides of the second strand are 13 nucleotides apart. In certain embodiments, the 5'-terminal and 3'-terminal nucleotides of the first strand are 19 nucleotides apart and the 5'-terminal and 3'-terminal nucleotides of the second strand are 13 nucleotides apart.

In certain embodiments, the 3'-terminal nucleotide of the second strand is complementary to a nucleotide of the first strand that is within 3 nucleotides from the 5'-terminal nucleotide of the first strand. In certain embodiments, the 3'-terminal nucleotide of the second strand is complementary to the 5'-terminal nucleotide of the first strand.

In certain embodiments, the 5'-terminal nucleotide of the second strand is complementary to a nucleotide of the first strand that is 1-4 nucleotides from the 3'-terminal nucleotide of the first strand. In certain embodiments, the 5'-terminal nucleotide of the second strand is complementary to a nucleotide of the first strand that is 1-2 nucleotides from the 3'-terminal nucleotide of the first strand.

In certain embodiments, at least one nucleotide of the sequence of 5' overhang is selected from the group consisting of A, U, and dT.

In certain embodiments, the GC content of the double stranded region is 20%-70%.

In certain embodiments, the first strand has a length from 19-22 nucleotides.

In certain embodiments, the first strand has a length of 21 nucleotides. In certain embodiments, the second strand has a length of 14-16 nucleotides.

In certain embodiments, the first strand has a length of 21 nucleotides, and the second strand has a length of 15 nucleotides. In certain embodiments, the first strand has a 3'-overhang of 2-4 nucleotides. In certain embodiments, the first strand has a 3'-overhang of 3 nucleotides. In certain embodiments, the first strand has a 5'-overhang of 3 nucleotides.

In certain embodiments, a duplex RNA molecule of the present disclosure, e.g. aiRNA, can contain at least one modified nucleotide or its analogue. In certain embodiments, the at least one modified nucleotide or its analogue can be a sugar-, backbone-, and/or base- modified ribonucleotide. In certain embodiments, the backbone-modified ribonucleotide can have a modification in a phosphodiester linkage with another ribonucleotide. In certain embodiments, the phosphodiester linkage is modified to include at least one of a nitrogen or a sulphur heteroatom. In another embodiment, the modified nucleotide or its analogue can be a backbone-modified ribonucleotide containing a phosphothioate group. In certain embodiments, the at least one modified nucleotide or its analogue is an unusual base or a modified base. In certain embodiments, the at least one modified nucleotide or its analogue comprises inosine, or a tritylated base. In certain embodiments, the modified nucleotide or its analogue is a sugar-modified ribonucleotide, wherein the 2'-OH group is replaced by a group selected from H, OR, R, halo, SH, SR, NH_2, NHR, NR_2, or CN, wherein each R is independently C_1-C_6 alkyl, alkenyl or alkynyl, and halo is F, Cl, Br, or I.

In certain embodiments, the first strand comprises at least one deoxynucleotide. In certain embodiments, the at least one deoxynucleotide is in one or more regions chosen from 3'-overhang, 5'-overhang, or double-stranded region. In another embodiment, the second strand comprises at least one deoxynucleotide.

In certain embodiments, a modulator of KRAS signaling comprises, for example, an aiRNA. In certain embodiments, a modulator of KRAS signaling comprises a KRAS-related

aiRNA. In certain embodiments, a modulator of KRAS signaling comprises a KRAS-specific aiRNA.

In certain embodiments, a modulator of KRAS signaling comprises, for example, one or more KRAS-specific aiRNAs that target one or more KRAS activating mutations as defined herein. In certain embodiments, a modulator of KRAS signaling comprises, for example, one or more KRAS-specific aiRNAs that target KRAS transcripts having one or more KRAS activating mutations at amino acid residues G12, G13, S17, P34, and/or Q61.

In certain embodiments, a modulator of KRAS signaling comprises, for example, one or more KRAS-specific aiRNAs that target both wild type and oncogenic KRAS RNA sequences.

Wild type KRAS-specific aiRNAs are described, for example, in the PCT International Application WO2015139044, the content of which is hereby incorporated by reference in its entirety for any purpose.

Exemplary KRAS aiRNA molecules, comprising a sense strand sequence, an antisense strand sequence or a combination of a sense strand sequence and antisense strand sequence are also shown in TABLE 4.

TABLE 4

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
1	CAGUUAUAGCUUAUU	320	AAUAAUAAGCUAUAACUGGCC	638
2	CCUAGUAGGAAAUAA	321	AAUUUAUUUCCUACUAGGACC	639
3	AGACCCAGUAUGAAA	322	AAAUUUCAUACUGGGUCUGCC	640
4	GUGCCAAGACAUUAA	323	AAAUUAAUGUCUUGGCACACC	641
5	CUCUUCUUCCAUAUU	324	AAUAAUAUGGAAGAAGAGUCC	642
6	AAUGGAAACUAUUAU	325	AAUAUAAUAGUUUCCAUUGCC	643
7	GUUGAUUACUUCUUA	326	AAAUAAGAAGUAAUCAACUGC	644
8	CUUAGCAAGAAGUUA	327	AAAUAACUUCUUGCUAAGUCC	645
9	CAGCACAAUCUGUAA	328	AAUUUACAGAUUGUGCUGAGC	646
10	CUUUCCACUGCUAUU	329	AAUAAUAGCAGUGGAAAGGAG	647
11	GGUGUGAAACAAAUU	330	AAUAAUUUGUUUCACACCAAC	648
12	UACAGCUAAUUCAGA	331	AAUUCUGAAUUAGCUGUAUCG	649
13	CUAAUUCAGAAUCAU	332	AAAAUGAUUCUGAAUUAGCUG	650
14	AAUUCAGAAUCAUUU	333	AAAAAAUGAUUCUGAAUUAGC	651
15	AUAUCAUCCAACAAU	334	AAUAUUGUUGGAUCAUAUUCG	652
16	GGUAACAGUAAUACA	335	AAAUGUAUUACUGUUACCAGG	653
17	CAGGACUUAGCAAGA	336	AAUUCUUGCUAAGUCCUGAGC	654
18	UGUGCCAGCUCUAUA	337	AAUUAUAGAGCUGGCACAGAG	655

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
19	CUAUAUUUACAUGCU	338	AAUAGCAUGUAAAUAUAGCCC	656
20	GUCUCUUGGAUAUUC	339	AAAGAAUAUCCAAGAGACAGG	657
21	GGGCUUUCUUUGUGU	340	AAUACACAAAGAAAGCCCUCC	658
22	GAUAUCUCCAUGAAG	341	AAACUUCAUGGAGAUAUCCAC	659
23	CAUUAUAGAGAACAA	342	AAUUUGUUCUCUAUAAUGGUG	660
24	CUGUAAUAUCUUACU	343	AAUAGUAAGAUAUUACAGACC	661
25	UGACGAUACAGCUAA	344	AAAUUAGCUGUAUCGUCAAGG	662
26	GAUGCUUUGAACAUC	345	AAAGAUGUUCAAAGCAUCAGC	663
27	CCCUGAUGAAUGUAA	346	AAUUUACAUUCAUCAGGGAUG	664
28	UAUUUGCCAUAAAUA	347	AAUUAUUUAUGGCAAAUACAC	665
29	AAAUAAUACUAAAUC	348	AAUGAUUUAGUAUUAUUUAUG	666
30	AAUCAUUUGAAGAUA	349	AAAUAUCUUCAAAUGAUUUAG	667
31	GAUAUUCACCAUUAU	350	AAUAUAAUGGUGAAUAUCUUC	668
W32	UUUAACAAAAGAUUU	351	AACAAAUCUUUUGUUAAACCA	669
W33	CCUAAUAUAUACAUA	352	AACUAUGUAUAUAUUAGGACA	670
W34	AAAAGAAACUGAAUA	353	AAGUAUUCAGUUUCUUUUUCA	671
35	AGCACAAUCUGUAAA	354	AAAUUUACAGAUUGUGCUGAG	672
36	CUUUCAUAGUAUAAC	355	AAAGUUAUACUAUGAAAGAGC	673
37	CUAGUGUGGUCUGUA	356	AAUUACAGACCACACUAGCAC	674
38	GUGUGGUCUGUAAUA	357	AAAUAUUACAGACCACACUAG	675
39	GACGUAUAUUGUAUC	358	AAUGAUACAAUAUACGUCUGC	676
40	CCCAAGUAGGCAUUC	359	AAAGAAUGCCUACUUGGGAAC	677
41	CGAAUAUGAUCCAAC	360	AAUGUUGGAUCAUAUUCGUCC	678
42	GCAAGUAGUAAUUGA	361	AAAUCAAUUACUACUUGCUUC	679
43	UCCUGAUGAUGAUUC	362	AAAGAAUCAUCAUCAGGAAGC	680
44	GACCUCAAGUGAUUC	363	AAUGAAUCACUUGAGGUCAGG	681
W45	UCCCUACCUUCCACA	364	AAAUGUGGAAGGUAGGGAGGC	682
W46	AUUUCCUUUUCACAU	365	AAAAUGUGAAAAGGAAAUGGC	683
W47	GUAUAUUGUAUCAUU	366	AAAAAUGAUACAAUAUACGUC	684
W48	CAUUUCCUUUUCACA	367	AAAUGUGAAAAGGAAAUGGCC	685
W49	GGAGAAUUCUAGAAA	368	AAAUUUCUAGAAUUCUCCCCC	686
50	CCCAAGUAGGCAUUC	369	AAAGAAUGCCUACUUGGGAAC	687
51	CGAAUAUGAUCCAAC	370	AAUGUUGGAUCAUAUUCGUCC	688
52	GCAAGUAGUAAUUGA	371	AAAUCAAUUACUACUUGCUUC	689
53	UCCUGAUGAUGAUUC	372	AAAGAAUCAUCAUCAGGAAGC	690
54	CUGUACUACUCCUAA	373	AAAUUAGGAGUAGUACAGUUC	691
55	GACCUCAAGUGAUUC	374	AAUGAAUCACUUGAGGUCAGG	692
56	CUUAGGUAGUGCUAG	375	AAACUAGCACUACCUAAGGAC	693
57	UCAGACUGCUCUUUC	376	AAUGAAAGAGCAGUCUGACAC	694
58	UGCUCUUUCAUAGUA	377	AAAUACUAUGAAAGAGCAGUC	695
59	GAUGAAUGUAAAGUU	378	AAUAACUUUACAUUCAUCAGG	696
60	GUCUGAUCCAUAUUU	379	AAUAAAUAUGGAUCAGACUUG	697
61	GACCAAAGAUGCUAA	380	AAUUUACCAUCUUUGCUCAUC	698
62	GAGGUGAAGUUUAUA	381	AAAUAUAAACUUCACCUCUUG	699
63	AGGGUGUUAAGACUU	382	AAUAAGUCUUAACACCCUACC	700
64	GGCAUCAUGUCCUAU	383	AAUAUAGGACAUGAUGCCUAG	701

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
65	UGAAUGUUCCCAAGU	384	AAUACUUGGGAACAUUCACUC	702
66	AGUAGGAAAUAAAUG	385	AAACAUUUAUUUCCUACUAGG	703
67	UGAACUAGUUCACAG	386	AAUCUGUGAACUAGUUCAGGC	704
68	GAAAUCUUCAUGCAA	387	AAAUUGCAUGAAGAUUUCUGG	705
69	GUGGAUAUCUCCAUG	388	AAUCAUGGAGAUAUCCACAGC	706
70	AGCAAGUAGUAAUUG	389	AAUCAAUUACUACUUGCUUCC	707
71	ACGUAUAUUGUAUCA	390	AAAUGAUACAAUAUACGUCUG	708
72	UUGACGAUACAGCUA	391	AAUUAGCUGUAUCGUCAAGGC	709
73	GGUGACUUAGGUUCU	392	AAUAGAACCUAAGUCACCUUC	710
74	UAGUUCUCUUAACAC	393	AAAGUGUUAAGAGAACUAGCC	711
75	UAUGUCAGAUAUUCA	394	AAAUGAAUAUCUGACAUACAC	712
76	CCUUUGAGCUUUCAU	395	AAUAUGAAAGCUCAAAGGUUC	713
77	ACAAGGAAACUUCUA	396	AAAUAGAAGUUUCCUUGUCUG	714
78	CGAUCAAGCUACUUU	397	AAUAAAGUAGCUUGAUCGAAG	715
79	UGCCAAUUUCUUACU	398	AAUAGUAAGAAAUUGGCACUC	716
80	GACAAAUCAAGAGCA	399	AAAUGCUCUUGAUUUGUCAGC	717
81	AUCUCAAACUCUUAG	400	AAACUAAGAGUUUGAGAUGAC	718
82	GAUGCCUUCUAUACA	401	AAAUGUAUAGAAGGCAUCAUC	719
83	GUAUGAAUAGACAGA	402	AAUUCUGUCUAUUCAUACCAG	720
84	UGAGUCACAUCAGAA	403	AAUUUCUGAUGUGACUCAGUG	721
85	GUCACCAUUGCACAA	404	AAAUUGUGCAAUGGUGACAAC	722
86	AAGCUCAGCACAAUC	405	AAAGAUUGUGCUGAGCUUGAC	723
87	AUUAUUAUAGCAACC	406	AAUGGUUGCUAUAAUAAUCCC	724
88	GGAAGAAGGUGACUU	407	AAUAAGUCACCUUCUUCCUAG	725
89	CUCUGAAGAUGUACC	408	AAAGGUACAUCUUCAGAGUCC	726
90	AGUAGCUGGGAUUAC	409	AAUGUAAUCCCAGCUACUCAG	727
91	GAGUUCUUGAAGAAU	410	AAUAUUCUUCAAGAACUCAUG	728
92	GACGAAUAUGAUCCA	411	AAUUGGAUCAUAUUCGUCCAC	729
93	UGGAUAUCUCCAUGA	412	AAUUCAUGGAGAUAUCCACAG	730
94	AAGGAAACUUCUAUG	413	AAACAUAGAAGUUUCCUUGUC	731
95	UAUAGCAGACGUAUA	414	AAAUAUACGUCUGCUAUAUUC	732
96	UCAAGCUACUUUAUG	415	AAACAUAAAGUAGCUUGAUCG	733
97	GGUAUGAAUAGACAG	416	AAUCUGUCUAUUCAUACCAGG	734
98	UAAUACAUUCCAUUG	417	AAACAAUGGAAUGUAUUACUG	735
99	AACCUUUGAGCUUUC	418	AAUGAAAGCUCAAAGGUUCAC	736
101	GAAAAUGACUGAAUA	419	AAAUAUUCAGUCAUUUUCAGC	737
102	AAAAUGACUGAAUAU	420	AAUAUAUUCAGUCAUUUUCAG	738
103	AAUGACUGAAUAUAA	421	AAUUUAUAUUCAGUCAUUUUC	739
104	UACAGCUAAAUUCAGA	422	AAUUCUGAAUUAGCUGUAUCG	740
105	CUAAUUCAGAAUCAU	423	AAAAUGAUUCUGAAUUAGCUG	741
106	AAUUCAGAAUCAUUU	424	AAAAAAUGAUUCUGAAUUAGC	742
107	UCAUUUUGUGGACGA	425	AAUUCGUCCACAAAAUGAUUC	743
108	AUAUGAUCCAACAAU	426	AAUAUUGUUGCAUCAUAUUCG	744
109	CCAACAAUAGAGGAU	427	AAAAUCCUCUAUUGUUGGAUC	745
110	CAAUAGAGGAUUCCU	428	AAUAGGAAUCCUCUAUUGUUG	746
111	UUCCUACAGGAAGCA	429	AAUUGCUUCCUGUAGGAAUCC	747

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
112	ACAGGAAGCAAGUAG	430	AAACUACUUGCUUCCUGUAGG	748
113	CAGGAAGCAAGUAGU	431	AAUACUACUUGCUUUCCUGUAG	749
114	GAAGCAAGUAGUAAU	432	AAAAUUACUACUUGCUUUCCUG	750
115	AGUAGUAAUUUCAUGG	433	AAUCCAUCAAUUACUACUUGC	751
116	GUAAUUGAUGGAGAA	434	AAUUUCUCCAUCAAUUACUAC	752
117	GGAGAAACCUGUCUC	435	AAAGAGACAGGUUUCUCCAUC	753
118	AAACCUGUCUCUUGG	436	AAUCCAAGAGACAGGUUUCUC	754
119	ACCUGUCUCUUGGAU	437	AAUAUCCAAGAGACAGGUUUC	755
120	UCUUGGAUAUUCUCG	438	AAUCGAGAAUAUCCAAGAGAC	756
121	UUGGAUAUUCUCGAC	439	AAUGUCGAGAAUAUCCAAGAG	757
122	GGAUAUUCUCGACAC	440	AAUGUGUCGAGAAUAUCCAAG	758
123	UAUUCUCGACACAGC	441	AAUGCUGUGUCGAGAAUAUCC	759
124	GACACAGCAGGUCAA	442	AACUUGACCUGCUGUGUCGAG	760
125	GGUCAAGAGGAGUAC	443	AAUGUACUCCUCUUGACCUGC	761
126	GAGGAGUACAGUGCA	444	AAUUGCACUGUACUCCUCUUG	762
127	GAGUACAGUGCAAUG	445	AAUCAUUGCACUGUACUCCUC	763
128	UGAGGGACCAGUAC	446	AAUGUACUGGUCCCUCAUUG	764
129	CCAGUACAUGAGGAC	447	AAAGUCCUCAUGUACUGGUCC	765
130	AGGGCUUUCUUUGUG	448	AAACACAAAGAAAGCCCUCCC	766
131	GGCUUUCUUUGUGUA	449	AAAUACACAAAGAAAGCCCUC	767
132	CUUUCUUUGUGUAUU	450	AAAAAUACACAAAGAAAGCCC	768
133	UGUGUAUUUGCCAUA	451	AAUUAUGGCAAAUACACAAAG	769
134	UUUGCCAUAAAUAA	452	AAAUUAUUUAUGGCAAAUAC	770
135	AAUCAUUUGAAGAUA	453	AAAUAUCUUCAAAUGAUUUAG	771
136	GAUAUUCACCAUUAU	454	AAUAUAAUGGUGAAUAUCUUC	772
137	UUAUAGAGAACAAAU	455	AAAAUUUGUUCUCUAUAAUGG	773
138	UAUAGAGAACAAAUU	456	AAUAAUUUGUUCUCUAUAAUG	774
139	AACAAAUUAAAAGAG	457	AAACUCUUUUAAUUUGUUCUC	775
140	CAAAUUAAAAGAGUU	458	AAUAACUCUUUUAAUUUGUUC	776
141	UUAAGGACUCUGAAG	459	AAUCUUCAGAGUCCUUAACUC	777
142	AAGGACUCUGAAGAU	460	AACAUCUUCAGAGUCCUUAAC	778
143	CUCUGAAGAUGUACC	461	AAAGGUACAUCUUCAGAGUCC	779
144	UCUGAAGAUGUACCU	462	AAUAGGUACAUCUUCAGAGUC	780
145	AAGAUGUACCUAUGG	463	AAACCAUAGGUACAUCUUCAG	781
146	AUGGUCCUAGUAGGA	464	AAUUCCUACUAGGACCAUAGG	782
147	UGGUCCUAGUAGGAA	465	AAUUUCCUACUAGGACCAUAG	783
148	CUAGUAGGAAAUAAA	466	AAAUUUAUUUCCUACUAGGAC	784
149	GGAAAUAAAUGUGAU	467	AAAAUCACAUUUAUUUCCUAC	785
150	UCUAGAACAGUAGAC	468	AAUGUCUACUGUUCUAGAAGG	786
151	GAACAGUAGACACAA	469	AAUUUGUGUCUACUGUUCUAG	787
152	CAGUAGACACAAAAC	470	AAUGUUUUGUGUCUACUGUUC	788
153	AACAGGCUCAGGACU	471	AAAAGUCCUGAGCCUGUUUUG	789
154	GCUCAGGACUUAGCA	472	AAUUGCUAAGUCCUGAGCCUG	790
155	UCAGGACUUAGCAAG	473	AAUCUUGCUAAGUCCUGAGCC	791
156	GACUUAGCAAGAAGU	474	AAAACUUCUUGCUAAGUCCUG	792
157	AGCAAGAAGUUAUGG	475	AAUCCAUAACUUCUUGCUAAG	793

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
158	AGAAGUUAUGGAAUU	476	AAGAAUUCCAUAACUUCUUGC	794
159	GUUAUGGAAUUCCUU	477	AAAAAGGAAUUCCAUAACUUC	795
160	AUGGAAUUCCUUUUA	478	AAAUAAAAGGAAUUCCAUAAC	796
161	AUUCCUUUUAUUGAA	479	AAUUUCAAUAAAAGGAAUUCC	797
162	ACAUCAGCAAAGACA	480	AAUUGUCUUUGCUGAUGUUUC	798
163	CAGCAAAGACAAGAC	481	AAUGUCUUGUCUUUGCUGAUG	799
164	AAGACAAGACAGGGU	482	AACACCCUGUCUUGUCUUUGC	800
165	AGACAAGACAGGGUG	483	AAACACCCUGUCUUGUCUUUG	801
166	AAGACAGGGUGUUGA	484	AAAUCAACACCCUGUCUUGUC	802
167	GACAGGGUGUUGAUG	485	AAUCAUCAACACCCUGUCUUG	803
168	GGUGUUGAUGAUGCC	486	AAAGGCAUCAUCAACACCCUG	804
169	GUUGAUGAUGCCUUC	487	AAAGAAGGCAUCAUCAACACC	805
170	UUGAUGAUGCCUUCU	488	AAUAGAAGGCAUCAUCAACAC	806
171	GAUGAUGCCUUCUAU	489	AAUAUAGAAGGCAUCAUCAAC	807
172	AUGCCUUCUAUACAU	490	AAAAUGUAUAGAAGGCAUCAU	808
173	GCCUUCUAUACAUUA	491	AACUAAUGUAUAGAAGGCAUC	809
174	UUCUAUACAUUAGUU	492	AAGAACUAAUGUAUAGAAGGC	810
175	CUAUACAUUAGUUCG	493	AAUCGAACUAAUGUAUAGAAG	811
176	UACAUUAGUUCGAGA	494	AAUUCUCGAACUAAUGUAUAG	812
177	GAAAUUCGAAAACAU	495	AAUAUGUUUUCGAAUUUCUCG	813
178	AAAUUCGAAAACAUA	496	AAUUAUGUUUUCGAAUUUCUC	814
179	AAACAUAAAGAAAAG	497	AAUCUUUUCUUUAUGUUUUCG	815
180	AACAUAAAGAAAAGA	498	AAAUCUUUUCUUUAUGUUUUC	816
181	AAAGAAAAGAUGAGC	499	AAUGCUCAUCUUUUCUUUAUG	817
182	AAGAUGGUAAAAAGA	500	AAUUCUUUUUACCAUCUUUGC	818
183	AGAUGGUAAAAAGAA	501	AACUUCUUUUUACCAUCUUUG	819
184	GGUAAAAAGAAGAAA	502	AAUUUUCUUCUUUUUACCAUC	820
185	AAAAAGAAGAAAAAG	503	AAUCUUUUUCUUCUUUUUACC	821
186	AAAAGAAGAAAAAGA	504	AAUUCUUUUUCUUCUUUUUAC	822
187	GAAAAAGAAGUCAAA	505	AACUUUGACUUCUUUUUCUUC	823
188	AAAGAAGUCAAAGAC	506	AAUGUCUUUGACUUCUUUUUC	824
189	GUCAAAGACAAAGUG	507	AAACACUUUGUCUUUGACUUC	825
190	AAAGACAAAGUGUGU	508	AAUACACACUUUGUCUUUGAC	826
191	AGACAAAGUGUGUAA	509	AAAUUACACACUUUGUCUUUG	827
192	AAAGUGUGUAAUUAU	510	AACAUAAUUACACACUUUGUC	828
193	AGUGUGUAAUUAUGU	511	AAUACAUAAUUACACACUUUG	829
194	UUUGUACUUUUUUCU	512	AAAAGAAAAAAGUACAAAUUG	830
195	CUUUUUUCUUAAGGC	513	AAUGCCUUAAGAAAAAAGUAC	831
196	UUUUCUUAAGGCAUA	514	AAGUAUGCCUUAAGAAAAAAG	832
197	AAGGCAUACUAGUAC	515	AAUGUACUAGUAUGCCUUAAG	833
198	ACUAGUACAAGUGGU	516	AAUACCACUUGUACUAGUAUG	834
199	GUACAAGUGGUAAUU	517	AAAAAUUACCACUUGUACUAG	835
200	CAAGUGGUAAUUUUU	518	AACAAAAAUUACCACUUGUAC	836
201	GUAAUUUUUGUACAU	519	AAAAUGUACAAAAAUUACCAC	837
202	AUUUUUGUACAUUAC	520	AAUGUAAUGUACAAAAAUUAC	838
203	CAUUACACUAAAUUA	521	AAAUAAUUUAGUGUAAUGUAC	839

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
204	UACACUAAAAUUAUUA	522	AACUAAUAAUUUAGUGUAAUG	840
205	AAUUAUUAGCAUUUG	523	AAACAAAUGCUAAUAAUUUAG	841
206	UUUGUUUUAGCAUUA	524	AAGUAAUGCUAAAACAAAUGC	842
207	UUAGCAUUACCUAAU	525	AAAAUUAGGUAAUGCUAAAAC	843
208	GCUCCAUGCAGACUG	526	AAACAGUCUGCAUGGAGCAGG	844
209	CUCCAUGCAGACUGU	527	AAAACAGUCUGCAUGGAGCAG	845
210	CCAUGCAGACUGUUA	528	AACUAACAGUCUGCAUGGAGC	846
211	UGCAGACUGUUAGCU	529	AAAAGCUAACAGUCUGCAUGG	847
212	UGUUAGCUUUUACCUU	530	AAUAAGGUAAAAGCUAACAGUC	848
213	AGCUUUUACCUUAAA	531	AAAUUUAAGGUAAAAGCUAAC	849
214	UUUACCUUAAAUGCU	532	AAAAGCAUUUAAGGUAAAAGC	850
215	UUACCUUAAAUGCUU	533	AAUAAGCAUUUAAGGUAAAAG	851
216	UUUUUUUCCUCUAAG	534	AAACUUAGAGGAAAAAAAAAC	852
217	GUAUUCCCAGAGUUU	535	AAAAAACUCUGGGAAUACUGG	853
218	AGUUUUGGUUUUUGA	536	AAUUCAAAAACCAAAACUCUG	854
219	UUUUGGUUUUUGAAC	537	AAAGUUCAAAAACCAAAACUC	855
220	GCAAUGCCUGUGAAA	538	AAUUUUCACAGGCAUUGCUAG	856
221	UGAAAAAGAAACUGA	539	AAUUCAGUUUCUUUUUCACAG	857
222	AAAAAGAAACUGAAU	540	AAUAUUCAGUUUCUUUUUCAC	858
223	AAUACCUAAGAUUUC	541	AAAGAAAUCUUAGGUAUUCAG	859
224	UACCUAAGAUUUCUG	542	AAACAGAAAUCUUAGGUAUUC	860
225	UUGAUUACUUCUUAU	543	AAAAUAAGAAGUAAUCAACUG	861
226	GAUUACUUCUUAUUU	544	AAAAAAUAAGAAGUAAUCAAC	862
227	UACUUCUUAUUUUUC	545	AAAGAAAAAUAAGAAGUAAUC	863
228	AUUUUUCUUACCAAU	546	AAAAUUGGUAAGAAAAAUAAG	864
229	ACCAAUUGUGAAUGU	547	AAAACAUUCACAAUUGGUAAG	865
230	UGUUGGUGUGAAACA	548	AAUUGUUUCACACCAACAUUC	866
231	UGAAACAAAUUAAUG	549	AAUCAUUAAUUUGUUUCACAC	867
232	AUUCUGUGUUUUAUC	550	AAAGAUAAAACACAGAAUAGG	868
233	UUCUGUGUUUUAUCU	551	AAUAGAUAAAACACAGAAUAG	869
234	AAAUGGAUUAAUUAC	552	AAAGUAAUUAAUCCAUUUAUG	870
235	CUAAUUGGUUUUUAC	553	AAAGUAAAAACCAAUUAGAAG	871
236	AUUGGUUUUUACUGA	554	AAUUCAGUAAAAACCAAUUAG	872
237	UUUACUGAAACAUUG	555	AAUCAAUGUUUCAGUAAAAAC	873
238	UCAUGUCCUAUAGUU	556	AAAAACUAUAGGACAUGAUGC	874
239	CACAAAGGUUUUGUC	557	AAAGACAAAACCUUUGUGAAC	875
240	AUUUCCUUUUCACAU	558	AAAAUGUGAAAAGGAAAUGGC	876
241	UUUCCUUUUCACAUU	559	AAUAAUGUGAAAAGGAAAUGG	877
242	CAUGCAGACUGUUAG	560	AAGCUAACAGUCUGCAUGGAG	878
243	GCAGACUGUUAGCUU	561	AAAAAGCUAACAGUCUGCAUG	879
244	GACUGUUAGCUUUUA	562	AAGUAAAAGCUAACAGUCUGC	880
245	UUAGCUUUUACCUUA	563	AAUUAAGGUAAAAGCUAACAG	881
246	UAAAUGCUUAUUUUA	564	AAUUAAAAUAAGCAUUUAAGG	882
247	AGUGGAAGUUUUUUU	565	AAAAAAAAAACUUCCACUGUC	883
248	GUGGAAGUUUUUUUU	566	AAAAAAAAAAACUUCCACUGU	884
249	CUAAGUGCCAGUAUU	567	AAGAAUACUGGCACUUAGAGG	885

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
250	AGUGCCAGUAUUCCC	568	AAUGGGAAUACUGGCACUUAG	886
251	CCAGUAUUCCCAGAG	569	AAACUCUGGGAAUACUGGCAC	887
252	AGUAUUCCCAGAGUU	570	AAAAACUCUGGGAAUACUGGC	888
253	GUAUUCCCAGAGUUU	571	AAAAAACUCUGGGAAUACUGG	889
254	UAUUCCCAGAGUUUU	572	AACAAAACUCUGGGAAUACUG	890
255	UUCCCAGAGUUUUGG	573	AAACCAAAACUCUGGGAAUAC	891
256	AGAGUUUUGGUUUUU	574	AACAAAAACCAAAACUCUGGG	892
257	GAGUUUUGGUUUUUG	575	AAUCAAAAACCAAAACUCUGG	893
258	GUUUUGGUUUUUGAA	576	AAGUUCAAAAACCAAAACUCU	894
259	UUGGUUUUUGAACUA	577	AACUAGUUCAAAAACCAAAAC	895
260	UUUUGAACUAGCAAU	578	AACAUUGCUAGUUCAAAAACC	896
261	CUAGCAAUGCCUGUG	579	AAUCACAGGCAUUGCUAGUUC	897
262	AUGCCUGUGAAAAAG	580	AAUCUUUUUCACAGGCAUUGC	898
263	UGCCUGUGAAAAAGA	581	AAUUCUUUUUCACAGGCAUUG	899
264	UGUGAAAAAGAAACU	582	AACAGUUUCUUUUUCACAGGC	900
265	GUGAAAAAGAAACUG	583	AAUCAGUUUCUUUUUCACAGG	901
266	AAAAGAAACUGAAUA	584	AAGUAUUCAGUUUCUUUUUCA	902
267	ACUGAAUACCUAAGA	585	AAAUCUUAGGUAUUCAGUUUC	903
268	UCUUGGGGUUUUUGG	586	AAACCAAAAACCCCAAGACAG	904
269	UUGGGGUUUUUGGUG	587	AAGCACCAAAAACCCCAAGAC	905
270	GGGGUUUUUGGUGCA	588	AAAUGCACCAAAAACCCCAAG	906
271	UGCAGUGUUUUUGGU	589	AACACCAAAAACACUGCAUGC	907
272	UUUGGUGCAUGCAGU	590	AAAACUGCAUGCACCAAAAAC	908
273	UUUCACAUUAGAUAA	591	AAUUUAUCUAAUGUGAAAAGG	909
274	UUCACAUUAGAUAAA	592	AAAUUUAUCUAAUGUGAAAAG	910
275	UGAAAUGGGGAUUAU	593	AAAAUAAUCCCCAUUUCAUAC	911
276	UUUUGGGGCUAUAUU	594	AAAAAUAUAGCCCCAAAAUGG	912
277	UUUGGGGCUAUAUUU	595	AAUAAAUAUAGCCCCAAAAUG	913
278	AAGAUUUUAACAAGU	596	AAUACUUGUUAAAAUCUUUUC	914
279	GUAUAAAAAAUUCUC	597	AAUGAGAAUUUUUUUAUACUUG	915
280	AGGAAUUAAAUGUAG	598	AAACUACAUUUAAUUCCUAUG	916
281	CUUUCAUAGUAUAAC	599	AAAGUUAUACUAUGAAAGAGC	917
282	UUUCAUAGUAUAACU	600	AAAAGUUAUACUAUGAAAGAG	918
283	UCAUAGUAUAACUUU	601	AAUAAAGUUAUACUAUGAAAG	919
284	UAAAUCUUUUCUUCA	602	AAUUGAAGAAAAGAUUUAAAG	920
285	CAACUUGAGUCUUUG	603	AAUCAAAGACUCAAGUUGAAG	921
286	CUUGAGUCUUUGAAG	604	AAUCUUCAAAGACUCAAGUUG	922
287	UCUUUGAAGAUAGUU	605	AAAAACUAUCUUCAAAGACUC	923
288	UUUGAAGAUAGUUUU	606	AAUAAAACUAUCUUCAAAGAC	924
289	UGAAGAUAGUUUUAA	607	AAAUUAAAACUAUCUUCAAAG	925
290	UUAUAGCUUAUUAGG	608	AAACCUAAUAAGCUAUAACUG	926
291	UAUUAGGUGUUGAAG	609	AAUCUUCAACACCUAAUAAGC	927
292	UUCAUAGAGAGUUUC	610	AAUGAAACUCUCUAUCAAAGC	928
293	GCAUUGGUUAGUCAA	611	AAUUUGACUAACCAAUGCAUG	929
294	UGCUUUUGUUUCUUA	612	AAUUAAGAAACAAAAGCAAUG	930
295	UUUGUUUCUUAAGAA	613	AAUUUCUUAAGAAACAAAAGC	931

aiRNA ID NO:	Sense Strand Sequence	Sense Strand SEQ ID NO:	Antisense Strand Sequence	Antisense Strand SEQ ID NO:
296	UUGUUUCUUAAGAAA	614	AAUUUUCUUAAGAAACAAAAG	932
297	AAGAAAACAAACUCU	615	AAAAGAGUUUGUUUUCUUAAG	933
298	AACAAACUCUUUUUU	616	AAUAAAAAAGAGUUUGUUUUC	934
299	UGAAGUGAAAAACUU	617	AAAAACUUUUUCACUUCAUUG	935
300	GUGAAAAAGUUUUAC	618	AAUGUAAAACUUUUUCACUUC	936
301	UUAACACUGGUUAAA	619	AAAUUUAACCAGUGUUAAGAG	937
302	AACACUGGUUAAAUU	620	AAUAAUUUAACCAGUGUUAAG	938
303	AAAUUAACAUUGCAU	621	AAUAUGCAAUGUUAAUUUAAC	939
304	UAAACACUUUUCAAG	622	AAACUUGAAAAGUGUUUAUGC	940
305	UCCUUUUGAUAAAUU	623	AAAAAUUUAUCAAAAGGAUUG	941
306	ACUUAGGUUCUAGAU	624	AAUAUCUAGAACCUAAGUCAC	942
307	UUAGGACUCUGAUUU	625	AAAAAAUCAGAGUCCUAAAAG	943
308	CACUUACUAUCCAUU	626	AAAAAUGGAUAGUAAGUGAUG	944
309	UUACUAUCCAUUUCU	627	AAAAGAAAUGGAUAGUAAGUG	945
310	ACUAUCCAUUUCUUC	628	AAUGAAGAAAUGGAUAGUAAG	946
311	UCCAUUUCUUCAUGU	629	AAAACAUGAAGAAAUGGAUAG	947
312	UUUCUUCAUGUUAAA	630	AAUUUUAACAUGAAGAAAUGG	948
313	GUCAUCUCAAACUCU	631	AAAAGAGUUUGAGAUGACUUC	949
314	CUCAAACUCUUAGUU	632	AAAAACUAAGAGUUUGAGAUG	950
315	AAACUCUUAGUUUUU	633	AAAAAAAACUAAGAGUUUGAG	951
316	UGUAAUUUAUAUUCC	634	AAUGGAAUAUAAAUUACAUAG	952
317	AAGGAUACACUUAUU	635	AAAAAUAAGUGUAUCCUUAUG	953
318	CAAUCUGUAAAUUUU	636	AAAAAAAUUUACAGAUUGUGC	954
319	UGUUACACCAUCUUC	637	AAUGAAGAUGGUGUAACAUAG	955

In certain embodiments, the RNA duplex molecule (aiRNA) comprises a sense strand sequence selected from the group consisting of SEQ ID NOs: 320-637. In certain embodiments, the RNA duplex molecule (aiRNA) comprises an antisense strand sequence selected from the group consisting of SEQ ID NOs: 638-955. In certain embodiments, the RNA duplex molecule (aiRNA) comprises a sense strand sequence selected from the group consisting of SEQ ID NOs: 320-637 and its corresponding complimentary antisense strand sequence as depicted in TABLE 4 above.

In certain embodiments, a KRAS RNA duplex molecule (aiRNA) comprises a sense strand sequence that is at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to a sequence selected from the group consisting of SEQ ID NOs: 320-637. In certain embodiments, the RNA duplex molecule (aiRNA) comprises an antisense strand sequence that is at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to a sequence selected from the group consisting of SEQ ID NOs: 638-955. In certain embodiments, the

RNA duplex molecule (aiRNA) comprises a sense strand sequence that is at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to a sequence selected from the group consisting of SEQ ID NOs: 320-637 and a substantially complimentary antisense strand sequence that independently is at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to the corresponding antisense strand as depicted in TABLE 4 above.

In certain embodiments, a modulator of KRAS signaling comprises, for example, one or more aiRNAs that target one or more effectors of the RAS/RAF/MEK/ERK/FRA signal transduction pathway. In certain embodiments, a modulator of KRAS signaling comprises, for example, one or more aiRNAs that target wildtype or mutant RAS GEFs. In certain embodiments, a modulator of KRAS signaling comprises, for example, one or more aiRNAs that target wildtype or mutant A-RAF, B-RAF and/or C-RAF/RAF-1. In certain embodiments, a modulator of KRAS signaling comprises, for example, one or more aiRNAs that target wildtype or mutant MEK 1/2. In certain embodiments, a modulator of KRAS signaling comprises, for example, one or more aiRNAs that target wildtype or mutant ERK 1/2. In certain embodiments, a modulator of KRAS signaling comprises, for example, one or more aiRNAs that target wildtype or mutant FRA-1.

The present disclosure also provides a method of preparing a duplex RNA molecule of the disclosure. In certain embodiments, the method comprises synthesizing the first strand and the second strand. In certain embodiments, the method comprises combining the synthesized strands under annealing conditions. In certain embodiments, the duplex RNA molecule is capable of effecting KRAS silencing. In certain embodiments, the RNA strands are chemically synthesized, or biologically synthesized. In certain embodiments, the first strand and the second strand are synthesized separately or simultaneously. In certain embodiments, the method comprises introducing at least one modified nucleotide or its analogue into the duplex RNA molecule during the synthesizing step, after the synthesizing and before the combining step, or after the combining step.

In certain embodiments, the present disclosure provides an expression vector for the generation of the duplex RNA *in vivo*. In certain embodiments, the vector comprises a nucleic acid or nucleic acids encoding the duplex RNA molecule operably linked to at least one expression-control sequence, e.g. a U6 snRNA promoter or an inducible promoter. In certain embodiments, the vector comprises a first nucleic acid encoding the first strand operably linked to a first expression-control sequence. In certain embodiments, the vector comprises a second

nucleic acid encoding the second strand operably linked to a second expression-control sequence. In another embodiment, the vector is a viral, eukaryotic, or bacterial expression vector. The present disclosure also provides a cell comprising the expression vector. In another embodiment, the cell comprises a duplex aiRNA molecule, e.g. a KRAS-specific aiRNA.

The present disclosure further provides a method of modulating KRAS signaling. In certain embodiments, the method comprises administering an asymmetrical duplex RNA molecule of the disclosure. In certain embodiments, the method comprises administering an asymmetrical duplex RNA molecule of the disclosure in an amount effective to silence KRAS expression or otherwise reduce KRAS expression. In certain embodiments, the method is in a cell or an organism. In certain embodiments, the method comprises contacting said cell or organism with an asymmetrical duplex RNA molecule of the disclosure, for example, under conditions wherein selective KRAS gene silencing can occur. In certain embodiments, the method comprises mediating a selective KRAS gene silencing affected by the duplex RNA molecule towards KRAS or nucleic acid having a sequence portion substantially corresponding to the double-stranded RNA. In certain embodiments, said contacting step comprises introducing said duplex RNA molecule into a target cell in culture or in an organism in which the selective KRAS silencing can occur. In certain embodiments, the introducing step is chosen from transfection, lipofection, electroporation, infection, injection, oral administration, inhalation, topical administration, or regional administration.

In certain embodiments, the introducing step comprises using a pharmaceutically acceptable excipient, carrier, or diluent. In certain embodiments, the pharmaceutically acceptable excipient, carrier, or diluent is chosen from a pharmaceutical carrier, a positive-charge carrier, a liposome, a protein carrier, a polymer, a nanoparticle, a nanoemulsion, a lipid, and a lipoid.

In certain embodiments, a modulator of KRAS signaling may comprise one or more inhibitors that target the KRAS activation of the RAF/MEK/ERK/FRA-1 signaling pathway. In certain embodiments, the method comprises administering an asymmetrical duplex RNA molecule of the disclosure in an amount effective in reducing (e.g., silencing) the KRAS activation of the RAF/MEK/ERK/FRA-1 signaling pathway.

In certain embodiments, a modulator of KRAS signaling may comprise one or more inhibitors that target the oncogenic KRAS activation of the RAF/MEK/ERK/FRA-1 signaling pathway. In certain embodiments, the method comprises administering an asymmetrical duplex

RNA molecule of the disclosure in an amount effective in reducing (e.g., silencing) the oncogenic KRAS activation of the RAF/MEK/ERK/FRA-1 signaling pathway.

In certain embodiments, a modulator of KRAS signaling may comprise an inhibitor of RAF activity (e.g. A-RAF, B-RAF, RAF1). In certain embodiments, a modulator of KRAS signaling may comprise an inhibitor of mitogen-activated kinase activity (e.g., MEK1, MEK2), an inhibitor of ERK activity (e.g., ERK1, ERK2). In certain embodiments, a modulator of KRAS signaling may comprise inhibitor of FRA-1 activity or any combination thereof. The skilled artisan will understand, however, that other downstream targets may also be suitable targets for an inhibitor of KRAS signaling, e.g. oncogenic KRAS signaling, and such targets are also encompassed by the present disclosure.

In certain embodiments, a modulator of KRAS signaling may comprise, for example, an RNA interfering agent that inhibits one or more downstream effectors of KRAS signaling.

In certain embodiments, a modulator of KRAS signaling may comprise an inhibitor of A-RAF, B-RAF, and/or C-RAF, for example, an A-RAF, B-RAF, and/or C-RAF RNA interfering agent (e.g., see WO2009143372, the content of which is hereby incorporated by reference in its entirety for any purpose).

In certain embodiments, a modulator of KRAS signaling may comprise an inhibitor of MEK activity, for example, a MEK RNA interfering agent (e.g., see published Patent Application No. 2009/0239936, the content of which is hereby incorporated by reference in its entirety for any purpose).

In certain embodiments, a modulator of KRAS signaling may comprise an inhibitor of ERK activity, for example, a ERK RNA interfering agent (e.g., see published Patent Application No. 2009/0239936, the content of which is hereby incorporated by reference in its entirety for any purpose).

In certain embodiments, a modulator of KRAS signaling may comprise an inhibitor of FRA-1 activity, for example, a FRA-1 RNA interfering agent (e.g. see U.S. Patent No. 6,124,133, the content of which is hereby incorporated by reference in its entirety for any purpose).

In certain embodiments, a modulator of KRAS signaling may comprise an antigen-binding protein or fragment thereof (e.g. a recombinant antigen-binding protein) that targets RAS protein, e.g. an oncogenic KRAS (e.g. see U.S. Patent Nos. 4,820,631; 5,084,380,

4,898,932; 5,081,230; 5,112,737; 5,028,527, the contents of which are hereby incorporated by reference in their entireties for any purpose), RAF protein (e.g. See WO2014047973, the content of which is hereby incorporated by reference in its entirety for any purpose), MEK protein, ERK protein and/or FRA-1 protein.

In certain embodiments, a modulator of KRAS signaling may comprise a small molecule inhibitor of KRAS signaling (see, for example, WO2016123378A1, the content of which is hereby incorporated by reference in its entirety for any purpose and TABLE 5 below).

TABLE 5

Exemplary inhibitors of the RAS/RAF/MEK/ERK signal transduction pathway [2]

INHIBITOR	TARGET(S)	CANCER EXAMINED	CLINICAL TRIALS	COMPANY
		RAS INHIBITORS		
Tipifarnib (Zarnestra™, R115777)	Ras, farnesyl-transferase, Rheb	AML, lymphoma, breast, glioma, melanoma	Phase I, II, III	Johnson & Johnson
		RAF INHIBITORS		
BAY 43-9006 (Nexavar®, sorafenib tosylate)	Raf, VEGFR2, VEGFR3, PDGF-R, c-Kit, c-Fms, Flt-3	renal cell carcinoma, HCC, melanoma, leukemias	Phase I, II, III	Bayer
AAI-881	Raf	thyroid, glioma	Preclinical	Novartis
IBT-613	Raf	glioma, thyroid	Preclinical	Novartis
RAF265	B-Raf, Raf-1 (c-Raf), A-Raf, B-Raf^{V600E}, VEGFR-2	melanoma	Phase I	Novartis
xl281	B-Raf, c-Raf, B- Raf^{V600E}	colorectal, papillary thyroid, ovarian, prostate, carcinoid	Phase I	Exelixis/Bristol Myers Squibb
sB-590885	Raf, B-RafV600E	melanoma	Preclinical	GlaxoSmithKline
Plx-4720	Raf, B-	melanoma	Preclinical	Plexxikon/Roche
Plx-4032	Raf, B-RafV600E	melanoma, thyroid, ovarian, solid tumors	Phase I	Plexxikon/Roche
l-779,450	Raf	leukemia	Preclinical	Merck

[2] modified from Chappell et al. Oncotarget. (2011) 2:135-64.

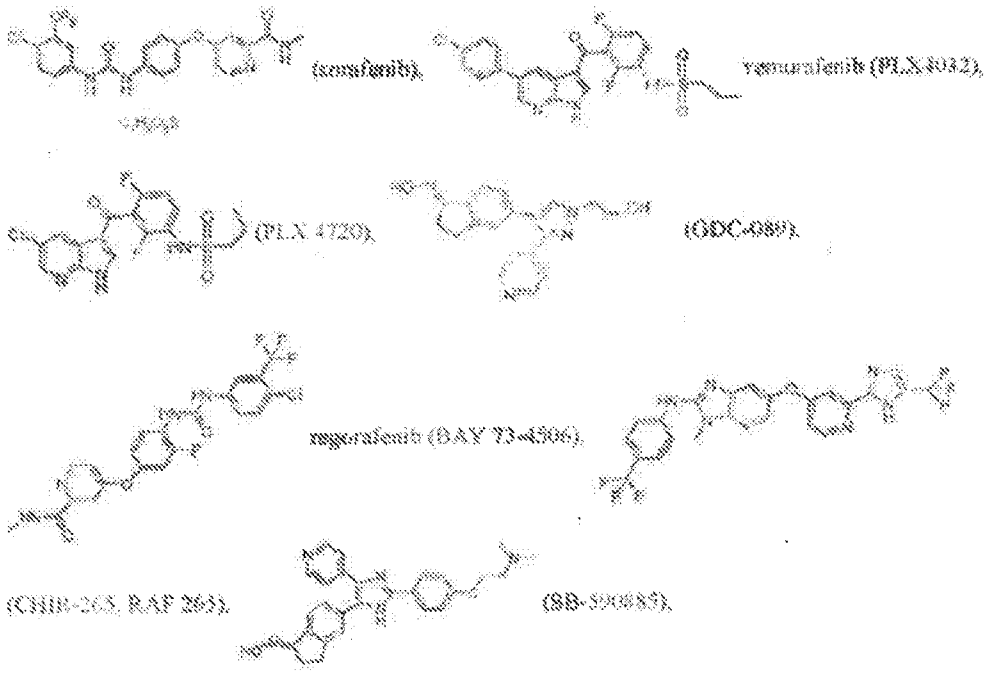
INHIBITOR	TARGET(S)	CANCER EXAMINED	CLINICAL TRIALS	COMPANY
Gw5074	Raf-1 (c-Raf)	melanoma, glioblastoma	Preclinical	GlaxoSmithKline
sB-699393	Raf		Preclinical	GlaxoSmithKline
MEK INHIBITORS				
CI-1040 (PD-184352)	MEK1, MKK5	colorectal, NSCLC, pancreatic, kidney,	Phase I, II (discontinued)	Pfizer
PD0325901	MEK1/2	breast, colon, NSCLC, melanoma	Phase I, II (discontinued)	Pfizer
xl518	MEK		Phase I	Exelixis
selumetinib (AZD6244, ARRY-142886)	MEK	melanoma, HCC, pancreatic, colon, lung, breast	Phase I, II	Astra Zeneca/Array BioPharma
RDEA119 (BAY 869766)	MAP2K1 (MAPK/ERK kinase 1)	advanced tumors	Phase I, II	Ardea/Bayer
PD098059	MEK1/2	advanced hematological and advanced solid cancers	Preclinical	Parke-Davis/Pfizer
u0126	MEK1/2	advanced hematological and advanced solid cancers	Preclinical	DuPont Pharmaceuticals
sl-327	MEK1/2	not evaluated for use in cancer treatment	Preclinical	DuPont Pharmaceuticals

In certain embodiments, a modulator of KRAS signaling may comprise a covalent inhibitor of KRAS G12C (see for example, WO2014152588 and WO2016049524, the contents of which are hereby incorporated by reference herein in their entireties for any purpose).

In certain embodiments, a modulator of KRAS signaling may comprise an inhibitor of prenyl transferase activity. In certain embodiments, a modulator of KRAS signaling comprises one or more agents each independently chosen from inhibitors of farnysyltransferase or geranylgeranyl transferase I (e.g., see U.S. Patent No. 5,965,539, the content of which is hereby incorporated by reference in its entirety for any purpose).

In certain embodiments, a modulator of KRAS signaling may comprise, for example, a small molecule RAF inhibitor. In certain embodiments, a small molecule RAF inhibitor can inhibit the RAF–MEK–ERK pathway signaling in cells expressing the BRAFV600E oncogene. Exemplary BRAF inhibitors include, but are not limited to, vemurafenib (RG7204 or PLX4032) which has been approved by the FDA for treatment of melanoma, dabrafenib which has been approved by FDA for treatment of cancers associated with a mutated version of the

gene BRAF, GDC-0879, PLX-4720 (Plexxikon/Roche) (R7204), Sorafenib Tosylate, dabrafenib and/or LGX818 or any combination thereof.



In certain embodiments, a modulator of KRAS signaling may comprise, for example, a small molecule MEK inhibitor. In certain embodiments, a MEK inhibitor may comprise, for example, an ATP-competitive MEK inhibitor, a non-ATP competitive MEK inhibitor, and/or an ATP-uncompetitive MEK inhibitor.

Exemplary MEK inhibitors include, but are not limited to, trametinib (GSK1120212; JTP 74057), for treatment of BRAF-mutated melanoma and possible combination with BRAF inhibitor dabrafenib (GSK2118436) to treat BRAF-mutated melanoma; selumetinib (ARRY-142886, AZD6244), for non-small cell lung cancer (NSCLC); binimetinib (MEK162, ARRY-162, ARRY-438162), for biliary tract cancer and melanoma; PD-325901, for breast cancer, colon cancer, and melanoma; Cobimetinib (GDC-0973; XL518); AS703026 (pimasertib), CI-1040, PD035901, RG7304, AZD6244, GSK1 120212, GDC-0973, XL-518, ARRY-162, ARRY-300, PD184161, RO4927350, CIP-1374, TAK-733, CH4987655, and/or RDEA1 19, or any combination thereof. In certain embodiments, the modulator of KRAS signaling may comprise, for example, RG7304, a small molecule MEK inhibitor with a structure based on a coumarin skeleton. It selectively inhibited RAF1 (C-RAF), B-RAF, mutant B-RAF (V600E),

and MEK1 in *in vitro* studies and showed a strong and broad spectrum of antitumor activities both *in vitro* in various tumor cell lines and *in vivo* in mouse xenograft models.

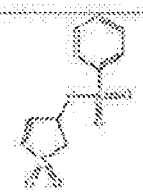
In certain embodiments, a modulator of KRAS signaling may comprise, for example, a non-ATP-competitive small- molecule MEK inhibitor (e.g. PD 098059, U0126, PD 184352 and its derivatives) or a biological inhibitor (e.g. anthrax lethal toxin and Yersinia outer protein J). In certain embodiments, a modulator of KRAS signaling may comprise, for example, a pyrrole derivative of MEK kinase. In certain embodiments, a modulator of KRAS signaling may comprise, for example, a 4-anilino-3-cyano-6,7-dialkoxyquinoline, including 4-anilino-3-cyano-6,7-dialkoxyquinolines (e.g., see WO2013059320, the content of which is hereby incorporated by reference in its entirety for any purpose).

In certain embodiments, a modulator of KRAS signaling may comprise, for example, a small molecule ERK inhibitor. Exemplary ERK inhibitors include, but are not limited to, SCH772984 which is an ATP-competitive ERK1 and ERK2 inhibitor, MK-8353/SCH900353, a clinical grade analogue of SCH772984, which is currently being tested in Phase I clinical trials, BVD-523 (Biomed Valley Discoveries) and RG7842 (GDC0994; Genentech/Roche) which is a selective inhibitor of ERK1/2. Phase I clinical trials evaluating RG7842 as a single agent or in combination with cobimetinib in solid tumors are ongoing. Studies conducted with SCH772984 indicate that the small molecule can inhibit cellular proliferation and cause apoptosis selectively in tumor cell lines that carry RAS or BRAF mutations, and also induce significant tumor regressions in mice with BRAF- or RAS-mutant xenografts. SCH772984 also demonstrated inhibition activity in cells that were resistant to either BRAF or MEK inhibitors and in cells that became resistant to the dual combination of these inhibitors.

In certain embodiments, a modulator of KRAS signaling may comprise, for example, a small molecule inhibitor with a thienyl benzenesulfonate scaffold that can inhibit ERK1/2 substrates containing an F-site or DEF (docking site for ERK, FXF) motif (e.g. see U.S. Patent No. 9,115,122, the content of which is incorporated by reference herein in its entirety for any purpose). In certain embodiments, a modulator of KRAS signaling may comprise, for example, an inhibitor of ERK2-mediated phosphorylation of c-FOS, e.g. SF-3-026 and its analogues. In certain embodiments, the modulator of KRAS signaling may comprise, for example, an inhibitor of AP-1 promoter activity in cells with constitutively active ERK1/2, e.g. SF-3-030 and its analogues.

Exemplary small molecule inhibitors of ERK1/2-regulated proteins include, but are not limited to the compounds depicted in TABLE 6.

TABLE 6

2.3.2 (SF-3-026)		(SF-3-029)	
(SF-3-027)		(SF-3-030)	

In certain embodiments, a modulator of KRAS signaling may comprise, for example, an antigen-binding protein or fragment thereof (e.g. a recombinant antigen-binding protein) that targets RAS protein. In certain embodiments, the RAS protein is chosen from an oncogenic KRAS (e.g., see U.S. Patent Nos. 4,820,631; 5,084,380, 4,898,932; 5,081,230; 5,112,737; 5,028,527, the contents of which are hereby incorporated by reference in their entireties for any purpose), RAF protein (e.g., see WO2014047973, the content of which is hereby incorporated by reference in its entirety for any purpose), MEK protein, ERK protein and/or FRA-1 protein.

In certain embodiments, a modulator of KRAS signaling may comprise, for example, a peptide that binds to and inhibits the activity of RAS protein. In certain embodiments, the RAS protein is chosen from RAF protein, MEK protein, ERK protein and/or FRA-1 protein. In certain embodiments, the peptide can be, for example, a chimeric peptide comprising a cell penetrating peptide, e.g. pro-apoptotic RAS and/or RAF peptides (see e.g. WO2015001045, the content of which is hereby incorporated by reference in its entirety for any purpose).

The present disclosure further provides compositions comprising a modulator of KRAS signaling. In certain embodiments, the composition is for the treatment of a KRAS-associated disorder or disease, e.g. cancer. In certain embodiments, the composition comprises a therapeutic agent.

In certain embodiments, the therapeutic agent can be, for example, a chemotherapeutic agent, a targeted agent, or an immunotherapeutic agent.

In certain embodiments, a modulator of KRAS signaling may be combined, for example, with an immunotherapeutic agent, e.g., any agent that can induce, enhance, or suppress an immune response in a subject.

In certain embodiments, an immunotherapeutic agent comprises an antibody or a recombinant antigen-binding protein or fragment thereof. In certain embodiments, a recombinant antigen-binding protein, or fragment thereof, can be, for example, monospecific, bispecific or multi-specific and monovalent or bivalent recombinant antigen-binding protein. In certain embodiments, an antigen-binding protein, or fragment thereof, can be an asymmetric bispecific antibody, an asymmetric bispecific IgG4, a CrossMab binding protein, a DAF (dual action Fab antibody; two-in-one), a DAF (dual action Fab antibody; four-in-one), a DutaMab, a DT-IgG, a knobs-in-holes binding protein, a Charge pair binding protein, a Fab-arm exchange binding protein, a SEEDbody, a Triomab (Triomab quadroma bispecific or removab bispecific), a LUZ-Y, a Fcab, a κλ-body, an iMab (innovative multimer), an Orthogonal Fab, a DVD-Ig binding protein, an IgG(H)-scFv, an scFv-(H)IgG, an IgG(L)-scFv, an scFv-(L)IgG, an IgG(L, H)-Fv, an IgG(H)-V, a V(H)-IgG, an IgG(L)-V, a V(L)-IgG, a KIH IgG-scFab, a 2scFv-IgG, an IgG-2scFv, an scFv4-Ig, a Zybody, a DVI-IgG (four-in-one), a nanobody (or VHH), a bispecific tandem nanobody, a bispecific trivalent tandem nanobody, a nanobody-HSA, a BiTE (bispecific T-cell engager) binding protein, a Diabody, a DART (dual affinity retargeting) binding protein, a TandAb (tetravalent bispecific tandem antibody), an scDiabody, an scDiabody-CH3, a Diabody-CH3, a Triple Body, a Miniantibody, a Minibody, a TriBi minibody, an scFv-CH3 KIH, a Fab-scFv, an scFv-CH-CL-scFv, a F(ab')2, a F(ab')2 scFv2, an scFv-KIH, a Fab-scFv-Fc, a Tetravalent HCAb, an scDiabody-Fc, a Diabody-Fc, a Tandem scFv-Fc, a Fabsc, a bsFc-1/2, a CODV-Ig (cross-over dual variable immunoglobulin), a biclonics antibody, an Intrabody, a Dock and Lock binding protein, an ImmTAC, an HSAbody, an scDiabody-HSA, a Tandem scFv-Toxin, an IgG-IgG binding protein, a Cov-X-Body, and/or an scFv1-PEG-scFv2. I.

In certain embodiments, an immunotherapeutic agent specifically binds to a specific cytokine, cytokine receptor, co-stimulatory molecule, co-inhibitory molecule, or immunomodulatory receptor that modulates the immune system. In certain embodiments, an immunotherapeutic agent specifically binds to a component of a regulatory T cell, myeloid suppressor cell, or dendritic cell.

In certain embodiments, an immunotherapeutic agent can be a cytokine, for example, an interferon (IFN), interleukin, or the like. Specifically, an immunotherapeutic agent can be interferon (IFNα or IFNβ), type 2 (IFNγ), or type III (IFNλ). An immunotherapeutic agent can also be interleukin-1 (IL-1), interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-11 (IL-11), interleukin-12 (IL-12), interleukin-13 (IL-13), or interleukin-18 (IL-18), or the like.

In certain embodiments, a modulator of KRAS signaling described herein may be combined, for example, with an immunotherapeutic agent. In certain embodiments, the immunotherapeutic agent targets and/or binds a cancer or tumor cell marker or component. Exemplary cancer or tumor cell markers or components include , but are not limited to, are not limited to, epidermal growth factor receptor (EGFR; EGFRl; ErbB-1; HERI); ErbB-2 (HER2/neu); ErbB-3/HER3; ErbB-4/HER4; EGFR ligand family; insulin-like growth factor receptor (IGFR) family; IGF-binding proteins (IGFBPs); IGFR ligand family (IGF-1R); platelet derived growth factor receptor (PDGFR) family; PDGFR ligand family; fibroblast growth factor receptor (FGFR) family; FGFR ligand family; vascular endothelial growth factor receptor (VEGFR) family; VEGF family; HGF receptor family; TRK receptor family; ephrin (EPH) receptor family; AXL receptor family; leukocyte tyrosine kinase (LTK) receptor family; TIE receptor family; angiopoietin 1; 2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family; discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor alpha (TGF-a); TGF-a receptor; Transforming growth factor-beta (TGF-β); TGF-β receptor; Interleukin β receptor alpha2 chain (IL13Ralpha2); Interleukin-6 (IL-6); 1L- 6 receptor; interleukin-4; IL-4 receptor; Cytokine receptors; Class I (hematopoietin family) and Class II (interferon/1 L- 10 family) receptors; tumor necrosis factor (TNF) family; TNF-a; tumor necrosis factor (TNF) receptor superfamily (TNTRSF); death receptor family; TRAIL- receptor; cancer-testis (CT) antigens; lineage-specific antigens; differentiation antigens; alpha-actinin-4; ARTC1; breakpoint cluster region-Abelson (Bcr-Abl) fusion products; B- RAF; caspase-5 (CASP-5); caspase-8 (CASP-8); beta-catenin (CTNNB1); cell division cycle 27 (CDC27); cyclin-dependent kinase 4 (CDK4); CDK 2A; COA-1; dek-can fusion protein; EFTUD-2; Elongation factor 2 (ELF2); Ets variant gene 6/acute myeloid leukemia 1 gene ETS (ETC6-AML1) fusion protein; fibronectin (FN); GPNMB; low density lipid receptor/GDP-L fucose: beta-D-Galactose 2-alpha-Lfucosyltraosferase (LDLR/FUT) fusion protein; HLA-A2; MLA-A11 ;

heat shock protein 70-2 mutated (HSP70-2M); KIAA0205; MART2; melanoma ubiquitous mutated 1; 2; 3 (MUM-1; 2; 3); prostatic acid phosphatase (PAP); neo-PAP; Myosin class 1; NFYC; OGT; OS-9; pml-RAR alpha fusion protein; PRDX5; PTPRK; K-RAS (KRAS2); N-RAS (NRAS); HRAS; RBAF600; SIRT12; SNRPD1; SYT-SSXl or -SSX2 fusion protein; triosephosphate isomerase; BAGE; BAGE-1; BAGE-2; 3; 4; 5; GAGE-1; 2; 3; 4; 5; 6; 7; 8; GnT-V (aberrant N-acetyl glucosaminyl transferase V; MGAT5); HERV-K MEL; KK-LC; KM-HN-1; LAGE; LAGE-1; CTL-recognized antigen on melanoma (CAMEL); MAGE-A1 (MAGE-1); MAGE-A2; MAGE- A3; MAGE-A4; MAGE- AS; MAGE-A6; MAGE-A8; MAGE-A9; MAGE-A10; MAGE-A11; MAGE-A12; MAGE-3; MAGE-B1; MAGE-B2; MAGE-B5; MAGE-B6; MAGE-C1; MAGE-C2; mucin 1 (MUC1); MART-I/Melan-A (MLANA); gp100; gp100/Pmel 17; tyrosinase (TYR); TRP-1; HAGE; NA-88; NY-ESO-1; NY-ESO-I/LAGE-2; SAGE; Spl7; SSX-1; 2; 3; 4; TRP2-1NT2; carcinoembryonic antigen (CEA); Kallikrein 4; mammaglobin-A; OA1; prostate specific antigen (PSA); prostate specific membrane antigen; TRP-1/; TRP-2 adipophilin; interferon inducible protein absent in melanoma 2 (AIM-2); BING-4; CPSF; cyclin DI; epithelial cell adhesion molecule (Ep-CAM); EpbA3; fibroblast growth factor-5 (FGF-5); glycoprotein 250 (gp250 intestinal carboxyl esterase (iCE); alpha-fetoprotein (AFP); M-CSF; mdm-2; MUCI; p53 (TP53); PBF; PRAME; PSMA; RAGE-1; RNF43; RU2AS; SOX10; STEAP1; survivin (BIRCS); human telomerase reverse transcriptase (hTERT); telomerase; Wilms' tumor gene (WT1); SYCP1; BRDT; SPANX; XAGE; ADAM2; PAGE-5; LIP1; CTAGE-1; CSAGE; MMA1; CAGE; BORIS; HOM-TES-85; AF15ql4; HCA66I; LDHC; MORC; SGY-1; SPO11; TPX1; NY-SAR-35; FTHLI7; NXF2 TDRD1; TEX 15; FATE; TPTE; immunoglobulin idiotypes; Bence-Jones protein; estrogen receptors (ER); androgen receptors (AR); CD40; CD30; CD20; CD19; CD33; CD4; CD25; CD3; cancer antigen 72-4 (CA 72-4); cancer antigen 15-3 (CA 15-3); cancer antigen 27-29 (CA 27-29); cancer antigen 125 (CA 125); cancer antigen 19-9 (CA 19-9); beta-human chorionic gonadotropin; 1-2 microglobulin; squamous cell carcinoma antigen; neuron-specific enolase; heat shock protein gp96; GM2; sargramostim; CTLA-4; 707 alanine proline (707-AP); adenocarcinoma antigen recognized by T cells 4 (ART-4); carcinoembryonic antigen peptide-1 (CAP-1); calcium- activated chloride channel-2 (CLCA2); cyclophilin B (Cyp-B); and/or human signet ring tumor-2 (HST-2).

In certain embodiments, a modulator of KRAS signaling described herein may be combined, for example, with one or more immunotherapeutic antibodies, each independently chosen from trastuzumab (anti- HER2/neu antibody); pertuzumab (anti-HER2 mAb);

cetuximab (chimeric monoclonal antibody to epidermal growth factor receptor EGFR); panitumumab (anti-EGFR antibody); nimotuzumab (anti-EGFR antibody); zalutumumab (anti-EGFR mAb); necitumumab (anti- EGFR mAb); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); rituximab (chimeric murine/human anti-CD20 mAb); obinutuzumab (anti-CD20 mAb); ofatumumab (anti-CD20 mAb); tositumumab-1131 (anti-CD20 mAb); ibritumomab tiuxetan (anti-CD20 mAb); bevacizumab (anti-VEGF mAb); ramucirumab (anti-VEGFR2 mAb); ranibizumab (anti-VEGF mAb); aflibercept (extracellular domains of VEGFR1 and VEGFR2 fused to IgGl Fc); AMG386 (angiopoietin-1 and -2 binding peptide fused to IgGl Fc); dalotuzumab (anti-IGF-lR mAb); gemtuzumab ozogamicin (anti-CD33 mAb); alemtuzumab (anti-Campath-l/CD52 mAb); brentuximab vedotin (anti-CD30 mAb): catumaxomab (bispecific mAb that targets epithelial cell adhesion molecule and CD3); naptumomab (anti-5T4 mAb); girentuximab (anti-Carbonic anhydrase ix); farletuzumab (anti-folate receptor); Panorex™ (17-1A) (murine monoclonal antibody); Panorex (@(17-1A)) (chimeric murine monoclonal antibody); BEC2 (ami-idiotypic mAb, mimics the GD epitope) (with BCG); oncolym (Lym-1 monoclonal antibody); SMART M195 Ab, humanized 13' 1 LYM-1 (Oncolym); Ovarex (B43.13, anti-idiotypic mouse mAb); 3622W94 mAb that binds to EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas; Zenapax (SMART Anti-Tac (IL-2 receptor); SMART Ml 95 Ab, humanized Ab, humanized); NovoMAb-G2 (pancarcinoma specific Ab); TNT (chimeric mAb to histone antigens); TNT (chimeric mAb to histone antigens); Gliomab-H (Monoclonals— Humanized Abs); GNI-250 Mab; EMD-72000 (chimeric-EGF antagonist); LymphoCide (humanized IL.L.2 antibody); or MDX-260 (bispecific, targets GD-2, ANA Ab, SMART IDIO Ab, SMART ABL 364 Ab), and/or ImmuRAIT-CEA.

In certain embodiments, an immunotherapeutic agent can be a cell, for example, an immune cell. For example, an immune cell, particularly one that is specific to a tumor, can be activated, cultured, and administered to a patient. The immune cell can be a natural killer cell, lymphokine-activated killer cell, cytotoxic T-cell, dendritic cell, or a tumor infiltrating lymphocyte (TIL). As used herein, tumor infiltrating lymphocytes (or TILs) refers to white blood cells ((i.e., T cells, B cells, NK cells, macrophages) that have left the bloodstream and migrated into a tumor. An analysis of patients with metastatic gastrointestinal cancers suggest $CD4^+$ and $CD8^+$ T cells within the TIL population are able to recognize neo-epitopes derived

from somatic mutations expressed by the patient's tumor. In certain embodiments, an immunotherapeutic agent can be, for example, sipuleucel-T (Provenge).

In certain embodiments, a modulator of KRAS signaling as disclosed herein may be combined with an inhibitor of an immune checkpoint.

Immune checkpoint proteins can regulate and maintain self-tolerance and the duration and amplitude of physiological immune responses including cytotoxic T cell activity.

Non-limiting examples of immune checkpoint proteins include cytotoxic T-lymphocyte-associated antigen (CTLA, for example, CTLA4) and its ligands CD 80 and CD86; programmed cell death protein (PD, for example, PD-1) and its ligands PD-L1 and PDL2; indoleamine-pyrrole 2,3-dioxygenase-1 (IDO1); T cell membrane protein (TIM, for example, TIM3); adenosine A2a receptor (A2aR); lymphocyte activation gene (LAG, for example, LAG3); killer immunoglobulin receptor (KIR); or the like.

As used herein, the term "immune checkpoint inhibitor" refers to a molecule that can completely or partially reduce, inhibit, interfere with, or modulate one or more immune checkpoint proteins that regulate T-cell activation or function. For example, the term "immune checkpoint inhibitor" can refer to a molecule that can interfere or/and prevent the interaction of PD-1 with its ligand, either PD-L1 or PD-L2.

In certain embodiments, the immune checkpoint inhibitor can target CTLA4. In certain embodiments, the immune checkpoint inhibitor can target PD-1. In certain embodiments, the immune checkpoint inhibitor can target PD-L1. In certain embodiments, the immune checkpoint inhibitor can target PD-L2. In certain embodiments, the immune checkpoint inhibitor can target LAG3. In certain embodiments, the immune checkpoint inhibitor can target B7-H3. In certain embodiments, the immune checkpoint inhibitor can target B7-H4. In certain embodiments, the immune checkpoint inhibitor can target TIM3.

In certain embodiments, the immune checkpoint inhibitor can be a small molecule.

In certain embodiments, the immune checkpoint inhibitor can be a small molecule that competes with an antibody or other antigen-binding protein, or fragment thereof, for binding to an immune checkpoint molecule.

In certain embodiments, the immune checkpoint inhibitor can be a small molecule that competes with an anti-PD-1 antibody, e.g., nivolumab, pembrolizumab, pidilizumab, BMS 936559, or atezolizumab as disclosed herein, for binding to PD-1.

In certain embodiments, the immune checkpoint inhibitor can be a small molecule that competes with an anti-PD-L1 antibody, e.g., atezolizumab, avelumab, or durvalumab as disclosed herein, for binding to PD-L1.

In certain embodiments, the immune checkpoint inhibitor can be a small molecule that competes with an anti-PD-L2 antibody, e.g., rHIgM12B7 or Dana-Farber patent anti-PD-L2 as disclosed herein, for binding to PD-L2.

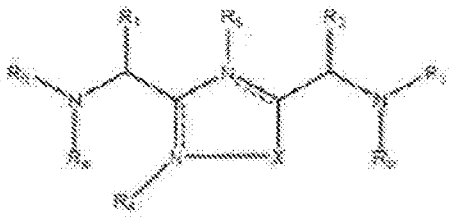
In certain embodiments, the immune checkpoint inhibitor can be a small molecule that competes with an anti-CTL4 antibody, e.g., ipilimumab, tremelimumab or *AGEN1884* as disclosed herein, for binding to CTL4.

In certain embodiments, the immune checkpoint inhibitor can be a small molecule that competes with an anti-VISTA antibody, e.g., the Janssen patent anti-VISTA antibody, Igenica patent anti-C10orf54 antibody or the Amplimmune patent anti-B7-H5 antibody as disclosed herein, for binding to the immune checkpoint protein, VISTA.

In certain embodiments, the immune checkpoint inhibitor can be a small molecule that rescues the inhibition of cell proliferation by recombinant PD-L1 in a mouse splenocyte assay by about 10% to about 95% (the mouse splenocyte assay is described in detail in, for example, WO2016142833).

In certain embodiments, the immune checkpoint inhibitor can be, for example, a 1,2,4-oxadiazole and thiadiazole compound, or a stereoisomer thereof, or a pharmaceutically acceptable salt thereof. In certain embodiments, the immune checkpoint inhibitor can suppress or inhibit the programmed cell death 1 (PD-1) signaling pathway in T cells.

In certain embodiments, the immune checkpoint inhibitor can be, for example, a compound of formula (I)



(I)

or a pharmaceutically acceptable salt or a stereoisomer thereof; wherein,

- - - - - is an optional double bond;

X is O or S;

R_1 and R_2 independently are a side chain of an amino acid or hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl or cycloalkyl; wherein (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl and cycloalkyl are optionally substituted by one or more substituents selected from amino, alkylamino, acylamino, carboxylic acid, carboxylate, carboxylic acid ester, thiocarboxylate, thioacid, -$CONR_7R_8$, hydroxy, cycloalkyl, (cycloalkyl)alkyl, aryl, arylalkyl, heterocyclyl, (heterocyclyl)alkyl, heteroaryl, (heteroaryl)alkyl, guanidino, -SH and -S(alkyl); optionally wherein cycloalkyl, aryl, heterocyclyl and heteroaryl are further substituted by one or more substituents such as hydroxy, alkoxy, halo, amino, nitro, cyano or alkyl and optionally wherein two or three carbon atoms of the (C_1-C_6)alkyl, (C_2-C_6)alkenyl or (C_2-C_6)alkynyl form part of a 3-7-membered carbocyclic or heterocyclic ring (such as a cyclobutyl or oxirane ring);

R_3 is hydrogen, -CO-$[Aaa1]_m$, $[Aaa1]_m$, $[Aaa1]_m$-CO-$[Aaa1]_m$, -$S(0)_p$-$[Aaa1]_m$, -$CONR_7R_8$, -COR_6, -$S0_2R_6$, (C_1-C_6)alkyl, (C_2-C_6)alkenyl or (C_2-C_6)alkynyl; wherein (C_1-C_6)alkyl, (C_2-C_6)alkenyl and (C_2-C_6)alkynyl are optionally substituted by one or more substituents selected from amino, alkylamino, acylamino, -COO-alkyl, carboxylic acid, carboxylate, thiocarboxylate, thioacid, -$CONR_7R_8$, hydroxy, aryl, arylalkyl, cycloalkyl, heterocyclyl, heteroaryl, (cycloalkyl)alkyl, (heterocyclyl)alkyl, (heteroaryl)alkyl, guanidino, -SH and -S(alkyl); optionally wherein cycloalkyl, aryl, heterocyclyl and heteroaryl are further substituted by one or more substituents such as hydroxy, alkoxy, halo, amino, nitro, cyano or alkyl, optionally wherein two or three carbon atoms of the (C_1-C_6)alkyl, (C_2-C_6)alkenyl or (C_2-C_6)alkynyl form part of a 3-7-membered carbocyclic or heterocyclic ring (such as a cyclobutyl or oxirane ring);

R_4 and R_5 independently are hydrogen or absent;

R_6 is hydrogen, alkyl, alkenyl, alkynyl, aralkyl, aryl, heteroaralkyl, heteroaryl, cycloalkyl, (cycloalkyl)alkyl, amino, aminoalkyl, hydroxyalkyl, alkoxyalkyl, acyl, $[Aaa2]_n$, -CO-$[Aaa2]_n$, $[Aaa2]_n$-CO-$[Aaa2]_n$ or -$S(0)_p$-$[Aaa2]_n$;

R_7 and R_8 independently are hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, aryl or heterocyclyl; wherein (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, aryl and heterocyclyl are optionally substituted by one or more substituents selected from halogen, hydroxyl, amino, nitro, cyano, cycloalkyl, heterocyclyl, heteroaryl, aryl, guanidino, (cycloalkyl)alkyl, (heterocyclyl)alkyl and (heteroaryl)alkyl; optionally wherein two or three

carbon atoms of the (C_1-C_6)alkyl, (C_2-C_6)alkenyl or (C_2-C_6)alkynyl form part of a 3-7-membered carbocyclic or heterocyclic ring (such as a cyclobutyl or oxirane ring);

alternatively R_7 and R_8 together with the nitrogen to which they are attached form an optionally substituted 3-7-membered ring containing 0-2 additional heteroatoms independently selected from N, O and S in any stable combination; wherein the optional substituent at each occurrence is selected from hydroxyl, -COOH, -COO-alkyl, amide, halo, amino, nitro and cyano;

[Aaa1] and [Aaa2], independently for each occurrence, represents an amino acid residue; wherein a C-terminal carboxyl group of amino acid residue is a free C-terminal carboxyl group (-COOH) or a modified C-terminal carboxyl group and an N-terminal amino group of amino acid residue is a free N-terminus (-NH_2) or a modified N-terminal amino group;

R_a is hydrogen or alkyl, alkenyl, alkynyl, acyl, aralkyl, aryl, heteroaralkyl, heteroaryl, cycloalkyl, (cycloalkyl)alkyl, aminoalkyl, hydroxyalkyl or alkoxyalkyl;

R_b is hydrogen, alkyl, alkenyl, alkynyl, acyl, aralkyl, aryl, heteroaralkyl, heteroaryl, cycloalkyl, (cycloalkyl)alkyl, aminoalkyl, hydroxyalkyl or alkoxyalkyl; or R_b and R_2, together with the atoms to which they are attached, may form pyrrolidine or piperidine optionally substituted with one or more groups independently selected from hydroxy, halo, amino, cyano and alkyl;

R_c is (C_1-C_6)alkyl, cycloalkyl, aryl, heterocyclcyl or heteroaryl; wherein the said (C_1-C_6)alkyl, cycloalkyl, aryl, heterocyclcyl or heteroaryl is optionally substituted by one or more substituents selected from carboxylic acid, hydroxy, alkyl, alkoxy, amino, alkylamino, acylamino, carboxylic ester, cycloalkyl, heterocyclyl, heteroaryl, (cycloalkyl)alkyl, (heterocyclyl)alkyl or (heteroaryl)alkyl;

m and n independently are integers selected from 1 to 3;

p is an integer selected from 1 to 2;

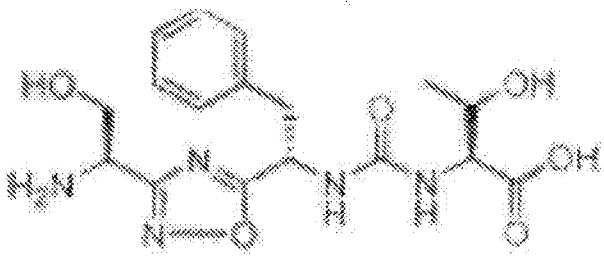
with a proviso that R_1 is not a side chain of Ser or Thr, when R_2 is a side chain of Asp, Asn, Glu or Gin, R_3 is hydrogen, -CO-Ser or -CO-Thr, R_6 is hydrogen, alkyl or acyl and R_a and R_b are hydrogen.

Compositions and methods of using and making the compounds of Formula (I) are disclosed in WO2016142833 (e.g. Compound Nos. 1-124 of WO2016142833), the content of which is hereby incorporated by reference herein in its entirety.

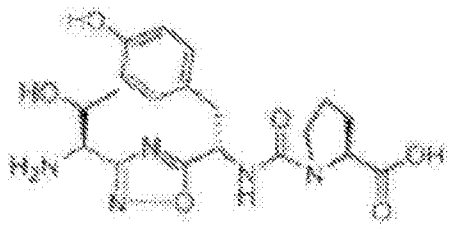
In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of a compound of Formula (I). In certain embodiments, the method is effective at sensitizing tumor cells to a compound of Formula (I). In certain embodiments, the method changes (e.g., enhances) the efficacy of a compound of Formula (I). In certain embodiments, the tumor cells are resistant to treatment with a compound of Formula (I) alone.

In certain embodiments, the compound of Formula (I) can be, for example, Compound No. 7 of WO2016142833, having the structure of:

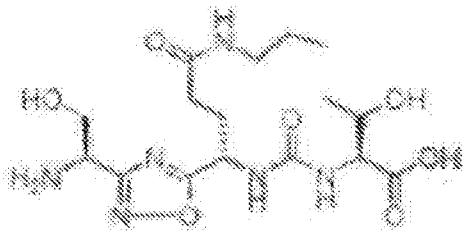
In certain embodiments, the compound of Formula (I) can be, for example, Compound No. 14 of WO2016142833, having the structure of:



In certain embodiments, the compound of Formula (I) can be, for example, Compound No. 60 of WO2016142833, having the structure of:

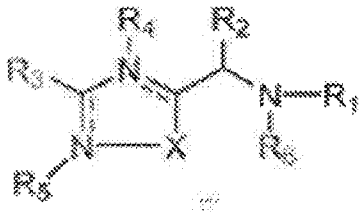


In certain embodiments, the compound of Formula (I) can be, for example, Compound No. 75 of WO2016142833, having the structure of:



In certain embodiments, the immune checkpoint inhibitor can be, for example, a 3-substituted 1,3,4-oxadiazole and thiadiazole compound, or a stereoisomer thereof, or a pharmaceutically acceptable salt thereof.

In certain embodiments, the immune checkpoint inhibitor can be, for example, a compound of formula (II):



(II)

or a pharmaceutically acceptable salt thereof or a stereoisomer thereof;

wherein,

X is O or S;

each dotted line [- - - - -] independently represents an optional bond;

R_1 is hydrogen or -CO-Aaa;

Aaa represents an amino acid residue;

R_2 is side chain of an amino acid, hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, heteroaralkyl, aralkyl, heteroaryl or aryl, each optionally substituted by one or more substituents selected from carboxylate, carboxylic acid, carboxylic acid ester, thiocarboxylate, thio acid, amido, amino, heterocyclyl, hydroxyl, cycloalkyl, aryl, aryl-COOH, heteroaryl, guanidino, amidino, -NH, -N(alkyl), -SH and -S(alkyl), optionally wherein two or three carbon atoms of the alkyl, alkenyl or alkynyl form part of a 3-7-membered carbocyclic or heterocyclic ring which is optionally substituted with 1 to 4 substituents, each independently selected from alkyl, alkoxy, carboxylic acid, carboxylate and hydroxyl;

R_3 is aryl, heteroaryl, heterocyclyl or cycloalkyl; wherein the said aryl, heteroaryl, heterocyclyl or cycloalkyl is optionally substituted by 1 to 4 occurrences of R_a;

R_a, independently for each occurrence, is alkyl, alkoxy, halo, hydroxyl, amino, -C(O)OH, aralkyl, aryl, alkoxy, heteroaralkyl, heteroaryl, cycloalkyl, (cycloalkyl)alkyl, hydroxyalkyl, alkoxyalkyl or acyl; or any two R_a groups attached to the same carbon atom together represent an oxo (=O) or thioxo (=S);

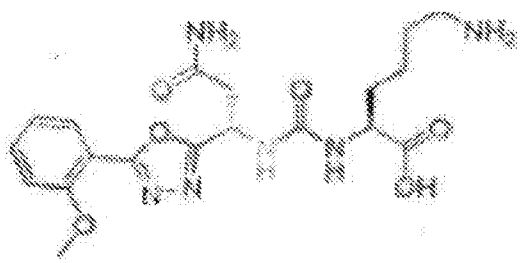
each of R_4 and R_5 independently is hydrogen or absent; and

R_6 is hydrogen or alkyl.

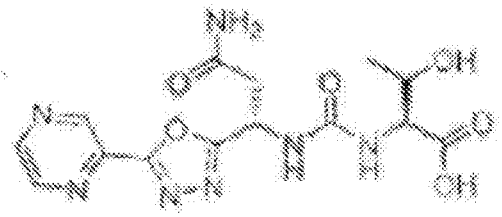
Compositions and methods of using and making the compounds of Formula (II are disclosed in WO2016142894 (see, for example, Compound Nos. 1- 30 of WO2016142894) and WO2016142886 (see, for example, Compound Nos. 1-62 of WO2016142886), the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of a compound of Formula (II). In certain embodiments, the method is effective at sensitizing tumor cells to a compound of Formula (II). In certain embodiments, the method changes (e.g., enhances) the efficacy of a compound of Formula (II). In certain embodiments, the tumor cells are resistant to treatment with a compound of Formula (II) alone.

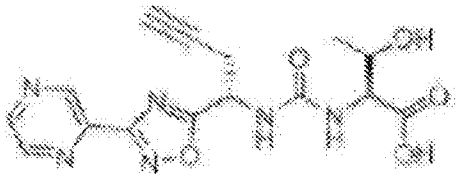
In certain embodiments, the compound of Formula (II) can be, for example, Compound No. 11 of WO2016142894, having the structure of:



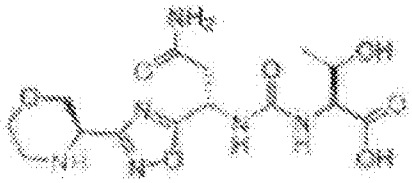
In certain embodiments, the compound of Formula (II) can be, for example, Compound No. 13 of WO2016142894, having the structure of:



In certain embodiments, the compound of Formula (II) can be, for example, Compound No. 32 of WO2016142886, having the structure of:

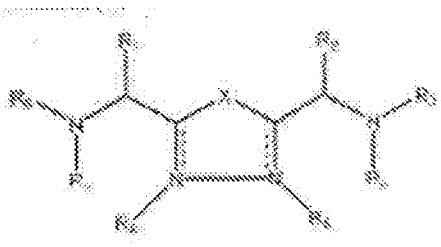


In certain embodiments, the compound of Formula (II) can be, for example, Compound No. 43 of WO2016142886, having the structure of:



In certain embodiments, the immune checkpoint inhibitor can be, for example, a 1,3,4-oxadiazole and thiadiazole compound or a stereoisomer thereof or a pharmaceutically acceptable salt thereof that can suppress or inhibit the programmed cell death 1 (PD- 1) signaling pathway.

In certain embodiments, the immune checkpoint inhibitor can be, for example, a compound of formula (III):



(III)

or a pharmaceutically acceptable salt thereof or a stereoisomer thereof; wherein, each dotted line [- - - -] independently represents an optional bond;

X is O or S;

R_1 and R_2 independently are a side chain of an amino acid or hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, heterocycloalkyl or cycloalkyl; wherein (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, heterocycloalkyl and cycloalkyl are optionally substituted by one or more substituents selected from amino, alkylamino, acylamino, carboxylic acid, carboxylate, carboxylic acid ester, thiocarboxylate, thioacid, -$CONR_7R_8$, hydroxy, cycloalkyl, (cycloalkyl)alkyl, aryl, heterocyclyl, (heterocyclyl)alkyl, heteroaryl, (heteroaryl)alkyl, guanidino, -SH and -S(alkyl); optionally wherein cycloalkyl, aryl, heterocyclyl and heteroaryl are further substituted by one or more substituents such as hydroxy, alkoxy, halo, amino, nitro, cyano or alkyl and optionally wherein two or three carbon atoms of the (C_1-C_6)alkyl, (C_2-C_6)alkenyl or (C_2-C_6)alkynyl form part of a 3-7-membered carbocyclic or heterocyclic ring (such as a cyclobutyl or oxirane ring);

R_3 is hydrogen, -CO-$[Aaa1]_m$, $[Aaa1]_m$, $[Aaa11]_m$-CO-$[Aaa1]_m$, -S(0)p-$[Aaa1]_m$, -$CONR_7R_8$, -COR_6, -SO_2R_6, (C_1-C_6)alkyl, (C_2-C_6)alkenyl or (C_2-C_6)alkynyl; wherein (C_1-C_6)alkyl, (C_2-C_6)alkenyl and (C_2-C_6)alkynyl are optionally substituted by one or more substituents selected from amino, alkylamino, acylamino, -COO-alkyl, carboxylic acid, carboxylate, thiocarboxylate, thioacid, - $CONR_7R_8$, hydroxy, aryl, arylalkyl, cycloalkyl, heterocyclyl, heteroaryl, (cycloalkyl)alkyl, (heterocyclyl)alkyl, (heteroaryl)alkyl, guanidino, -SH and -S(alkyl); optionally wherein cycloalkyl, aryl, heterocyclyl and heteroaryl are further substituted by one or more substituents such as hydroxy, alkoxy, halo, amino, nitro, cyano or alkyl, optionally wherein two or three carbon atoms of the (C_1-C_6)alkyl, (C_2-C_6)alkenyl or (C_2-C_6)alkynyl form part of a 3-7-membered carbocyclic or heterocyclic ring (such as a cyclobutyl or oxirane ring);

R_4 and R_5 independently are hydrogen or absent;

R_6 is hydrogen, alkyl, alkenyl, alkynyl, aralkyl, aryl, heteroaralkyl, heteroaryl, cycloalkyl, (cycloalkyl)alkyl, amino, aminoalkyl, hydroxyalkyl, alkoxyalkyl, acyl, $[Aaa2]_n$, -CO-$[Aaa2]_n$, $[Aaa2]_n$-CO-$[Aaa2]_n$ or -$S(O)_p$-$[Aaa2]_n$;

R_7 and R_8 independently are hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, aryl, cycloalkyl or heterocyclyl; wherein (C_1-C_6)alkyl, (C_2-C_6)alkenyl and (C_2-C_6)alkynyl, aryl and heterocyclyl are optionally substituted by one or more substituents selected from halogen, hydroxyl, amino, nitro, cyano, cycloalkyl, heterocyclyl, heteroaryl, aryl, guanidino, (cycloalkyl)alkyl, (heterocyclyl)alkyl and (heteroaryl)alkyl; optionally wherein two or three

carbon atoms of the (C_1-C_6)alkyl, (C_2-C_6)alkenyl or (C_2-C_6)alkynyl form part of a 3-7-membered carbocyclic or heterocyclic ring (such as a cyclobutyl or oxirane ring);

alternatively, R_7 and R_8 together with the nitrogen to which they are attached form an optionally substituted 3-7-membered ring containing 0-2 additional heteroatoms independently selected from N, O and S in any stable combination; wherein the optional substituent at each occurrence is selected from hydroxyl, -COOH, -COO-alkyl, amide, halo, amino, nitro and cyano;

[Aaa1] and [Aaa2], independently for each occurrence, represents an amino acid residue; wherein a C-terminal carboxyl group of amino acid residue is a free C-terminal carboxyl group (-COOH) or a modified C-terminal carboxyl group and an N-terminal amino group of amino acid residue is a free N-terminus (-NH_2) or a modified N-terminal amino group;

R_a is hydrogen or alkyl, alkenyl, alkynyl, acyl, aralkyl, aryl, heteroaralkyl, heteroaryl, cycloalkyl, (cycloalkyl)alkyl, aminoalkyl, hydroxyalkyl or alkoxyalkyl; or R_a and R_2, together with the atoms to which they are attached, form heterocycloalkyl ring optionally substituted with one or more groups independently selected from hydroxyl, halo, amino, cyano and alkyl;

R_b is hydrogen or alkyl, alkenyl, alkynyl, acyl, aralkyl, aryl, heteroaralkyl, heteroaryl, cycloalkyl, (cycloalkyl)alkyl, aminoalkyl, hydroxyalkyl or alkoxyalkyl;

R_c is (C_1-C_6)alkyl, cycloalkyl, aryl, heterocyclyl or heteroaryl; wherein the said (C_1-C_6)alkyl, cycloalkyl, aryl, heterocyclyl or heteroaryl is optionally substituted by one or more substituents selected from carboxylic acid, hydroxy, alkyl, alkoxy, amino, alkylamino, acylamino, carboxylic ester, cycloalkyl, heterocyclyl, heteroaryl, (cycloalkyl)alkyl, (heterocyclyl)alkyl or (heteroaryl)alkyl;

m and n independently are integers from 1 to 3; and

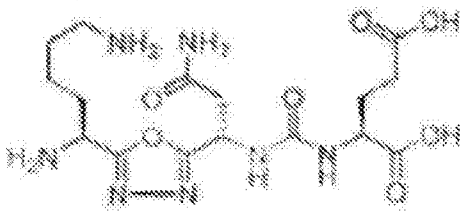
p is an integer selected from 1 to 2;

with a proviso that R_1 is not a side chain of Ser, Thr, Phe, Ala or Asn, when R_2 is side chain of Ser, Ala, Glu, Gln, Asn or Asp, R_3 is hydrogen, -CO-Ser, -CO-Thr or -CO-Asn and R_a, R_b and R_6 are hydrogen.

Compositions and methods of using and making the compounds of Formula (I) are disclosed in WO2016142852 (e.g. Compound Nos. 1-55 of WO2016142852), the content of which is hereby incorporated by reference herein in its entirety.

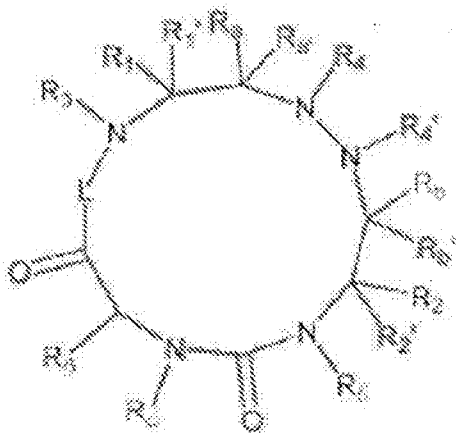
In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of a compound of Formula (III). In certain embodiments, the method is effective at sensitizing tumor cells to a compound of Formula (III). In certain embodiments, the method changes (e.g., enhances) the efficacy of a compound of Formula (III). In certain embodiments, the tumor cells are resistant to treatment with a compound of Formula (III) alone.

In certain embodiments, the compound of Formula (III) can be, for example, Compond No. 1 having the structure of:



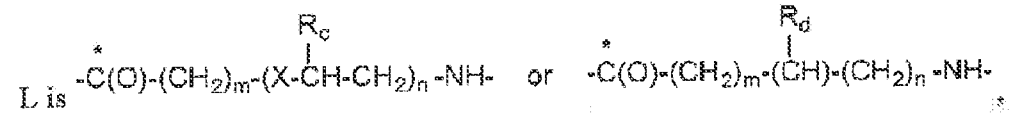
In certain embodiments, the immune checkpoint inhibitor can be, for example, a cyclic compound of formula (IV) or a stereoisomer thereof or a pharmaceutically acceptable salt thereof that can suppress or inhibit the programmed cell death 1 (PD- 1) signaling pathway.

In certain embodiments, the immune checkpoint inhibitor can be, for example, a compound of formula (IV):



(IV)

or a pharmaceutically acceptable salt thereof or a stereoisomer thereof; wherein,

L is  or

wherein the -C(O)- group marked with * is connected to the nitrogen bearing R_3 in Formula (IV);

X is CH_2, O, NH or S;

R_1, R_2 and R_6 independently are a side chain of an amino acid, hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl or (C_2-C_6)alkynyl; wherein (C_1-C_6)alkyl, (C_2-C_6)alkenyl and (C_2-C_6)alkynyl are optionally substituted by one or more substituents selected from hydroxy, amino, amido, alkylamino, acylamino, -$(CH_2)_m$-COOH, -$(CH_2)_m$-COO-alkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, guanidino, (cycloalkyl)alkyl, (heterocyclyl)alkyl, (heteroaryl)alkyl, -SH and -S-(alkyl); optionally wherein cycloalkyl, aryl, heterocyclyl and heteroaryl are further substituted optionally by one or more substituents such as hydroxy, alkoxy, halo, amino, nitro, cyano or alkyl; optionally wherein two or three carbon atoms of the (C_1-C_6)alkyl, (C_2-C_6)alkenyl or (C_2-C_6)alkynyl form part of a 3-7-membered carbocyclic or heterocyclic ring (such as a cyclobutyl or oxirane ring);

R_1', R_2', R_3 and R_5 independently are hydrogen or alkyl;

or R_1 and R_1', together with the carbon atom to which they are attached, may optionally form an optionally substituted cycloalkyl or heterocycloalkyl ring;

or R_1 and R_3, together with the atoms to which they are attached, may optionally form a heterocyclic ring optionally substituted with one or more groups independently selected from amino, cyano, alkyl, halo and hydroxy;

or R_2 and R_2', together with the carbon atom to which they are attached, may optionally form an optionally substituted cycloalkyl or heterocycloalkyl ring;

or R_2 and R_5, together with the atoms to which they are attached, may optionally form a heterocyclic ring optionally substituted with one or more groups independently selected from amino, cyano, alkyl, halo and hydroxy;

R_4 and R_4' independently are hydrogen or alkyl;

R_a and R_a' are each hydrogen; or together represent an oxo (=O) group;

R_b and R_b' are each hydrogen; or together represent an oxo (=O) group;

R_c at each occurrence is independently hydrogen or alkyl;

R_d is amino or -NH-C(O)-$(CH_2)_r$-CH_3;

m is an integer from 0 to 3;

n, independently for each occurrence, is an integer from 2 to 20;

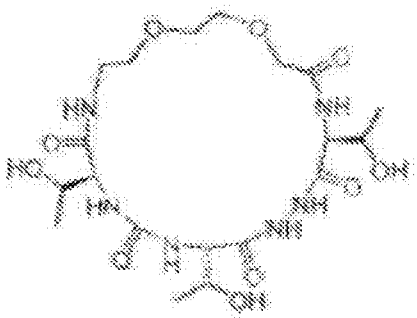
r, is an integer from 0-20; and

with a proviso that R_6 is not a side chain of Ser, Asp, Ala, He, Phe, Trp, Lys, Glu and Thr, when R_1 is a side chain of Ala, Ser, Thr or Leu, R_2 is a side chain of Asp, Asn, Glu or Gln and R_5 and R_c are hydrogen.

Compositions and methods of using and making the compounds of Formula (I) are disclosed in WO2016142835 (e.g. Compound Nos, 1-20 of WO2016142835), the content of which is hereby incorporated by reference herein in its entirety.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of a compound of Formula (IV). In certain embodiments, the method is effective at sensitizing tumor cells to a compound of Formula (IV). In certain embodiments, the method changes (e.g., enhances) the efficacy of a compound of Formula (IV). In certain embodiments, the tumor cells are resistant to treatment with a compound of Formula (IV) alone.

In certain embodiments, the compound of Formula (IV) can be, for example, the compound No. 12 having the structure of:

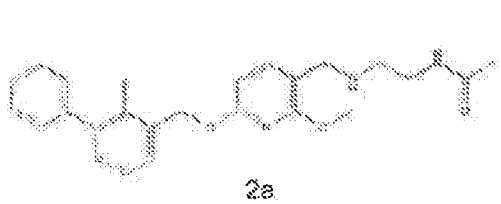


In certain embodiments, the immune checkpoint inhibitor can be, for example, a peptidomimetic compound that inhibits the immunosuppressive signal induced by an immune checkpoint, e.g. PD-1, PD-L1, PD-L2, CTL-4 and/or VISTA. Non-limiting exemplary peptidomimetic compounds are disclosed in U.S. Patent Nos. 8,907,053; 9,044,442; 9,096,642; 9,233,940 and U.S. Patent Publication Nos. 2015/0087581; 2015/0125491; 2016/0113901 and 2016/0194295, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

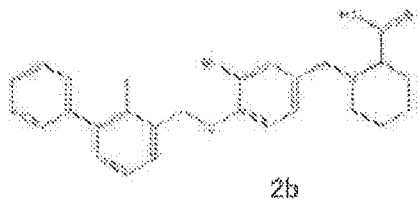
In certain embodiments, the small molecule immune checkpoint inhibitor can be, for example, CA-170 (previously AUPM 170), a first-in-class oral, small molecule antagonist that selectively targets PD-L1, PD-L2 and V-domain Ig suppressor of T cell activation (VISTA) immune checkpoints CA-170 is currently being evaluated in a phase I trial for the treatment of advanced solid tumors or lymphomas (see Abstract 4861, AACR 107th Annual Meeting 2016; April 16-20, 2016; New Orleans, LA; developed by Curis and Aurigene).

In certain embodiments, the small molecule immune checkpoint inhibitor can be, for example, one or more of the Bristol-Myers Squibb (BMS) compounds based on the (2-methyl-3-biphenylyl) methanol scaffold disclosed in WO2015034820, the content of which is hereby incorporated by reference herein in its entirety.

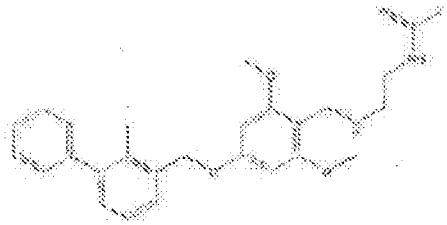
In certain embodiments, he small molecule immune checkpoint inhibitors can be, for example, compounds 8, 37, 202 and 242 of WO2015034820 (designated herein as BMS-8, BMS-37, BMS-202 and BMS-242) and having the structure of:



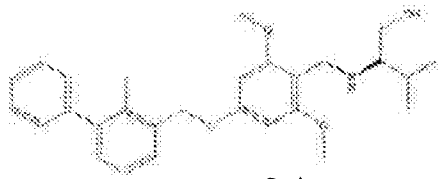
2a
(BMS-202)



2b
(BMS-8)



2c
(BMS-37)



2c1
(BMS-242)

BMS-8, BMS-37, BMS-202 and BMS-242 bind directly to PD-L1 and not PD-1 and effectively dissociate a preformed PD-1/PD-L1 complex *in vitro*. NMR studies indicate these molecules block PD-1/PD-L1 interaction by inducing PD-L1 dimerization through PD-1 interacting surface.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of BMS-202. In certain embodiments, the method is effective at sensitizing tumor cells to BMS-202. In certain embodiments, the method changes (e.g., enhances) the efficacy of BMS-202. In certain embodiments, the tumor cells are resistant to treatment with BMS-202 alone.

In certain embodiments, the small molecule immune checkpoint inhibitor can be, for example, an immune checkpoint-specific peptide aptamer.

In certain embodiments, the small molecule immune checkpoint inhibitor can be, for example, an immune checkpoint-specific affimer.

Affimers are peptide aptamers that are engineered into a modified human protease inhibitor Stefin A scaffold (see, for example, U.S. Patent No. 9,447,170, the content of which is hereby incorporated by reference herein in its entirety for any purpose).

In certain embodiments, the small molecule immune checkpoint inhibitor may comprise an amino acid sequence having at least 80% identity to the Stefin A scaffold polypeptide sequence of SEQ ID NO.: 987.

MIPGGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVVAG

TNYYIKVRAGDNKYMHLKVFKSLPGQNEDLVLTGYQVDKNKDDELTGF

(SEQ ID NO.: 987)

In certain embodiments, the small molecule immune checkpoint inhibitor may comprise an amino acid sequence having at least 80% identity to the modified Stefin A scaffold polypeptide sequence of SEQ ID NO.: 988.

MIPWGLSEAKPATPEIQEIVDKVKPQLEEKTNETYGKLEAVQYKTQVDAG

TNYYIKVRAGDNKYMHLKVFNGPPGQNEDLVLTGYQVDKNKDDELTGF

(SEQ ID NO.: 988)

In certain embodiments, the small molecule immune checkpoint inhibitor can be, for example, a PD-L1-specific affimer (e.g. PDL1-141 or PDL1-179; see, for example, Avacta Life Sciences poster entitled "Generation and Formatting of Affimer® Biotherapeutics for the Inhibition of the PD-L1/PD-1 Pathway" 14th Annual Discovery on Target, September 19-22, 2016, Boston, MA).

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of a PD-L1-specific affimer. In certain embodiments, the method is effective at sensitizing tumor cells to PD-L1-specific affimer. In certain embodiments, the method changes (e.g., enhances) the efficacy of PD-L1-specific affimer. In certain embodiments, the tumor cells are resistant to treatment with PD-L1-specific affimer alone. In certain embodiments, the PD-L1-specific affimer can comprise PDL1-141 and/or PDL1-179.

In certain embodiments, the small molecule immune checkpoint inhibitor can be, for example, a bromodomain and extraterminal domain (BET) inhibitor e.g., JQ1 (also known as TEN-010 in clinical trials NCT02308761 and NCT01987362). In certain embodiments, a bromodomain and extraterminal domain (BET) inhibitor can be an inhibitor of BRD4.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of a BET inhibitor. In certain embodiments, the method is effective at sensitizing tumor cells to BET inhibitor. In certain embodiments, the method changes (e.g., enhances) the efficacy of BET inhibitor. In certain embodiments, the tumor cells are resistant to treatment with BET inhibitor alone. In certain embodiments, the BET inhibitor can comprise an inhibitor of BRD4, e.g. JQ1.

In certain embodiments, the immune checkpoint inhibitor is a monoclonal or polyclonal antibody directed at PD-1 , PD-L1, PDL2, CD28, CD80, CD86, CTLA4, B7RP1, ICOS, B7RPI, B7-H3, B7-H4, BTLA, HVEM, KIR, TCR, LAG3, CD137, CD137L, OX40, OX40L, CD27, CD70, CD40, CD40L, TIM3 (i.e., HAVcr2), GAL9, and/or A2aR.

Exemplary PD-1 immune checkpoint inhibitors that may be combined with a modulator of KRAS signaling, as disclosed herein, include, but are not limited, to:

Nivolumab

Synonym: Anti-PD-1 human monoclonal antibody MDX-1106

Description: A fully human IgG4 antibody blocking the programmed cell death-1 receptor (Medarex/Ono Pharmaceuticals/Bristol-Myers Squibb) gamma1 heavy chain (1-440) [Homo sapiens VH (IGHV3 -33*01 (91.80%) – (IGHD) – IGHJ4*01) [8.8.6] (1-113) – IGHG4*01 hinge S10>P (221) (114-440)], (127-214') – disulfide with kappa light chain (1'-214') [Homo sapiens V- KAPPA (IGKV3- 11*01 (98.90%) – IGKJ1*01) [6.3.9] (1'-107') – IGKC*01 (108'-214')]; (219-219'':222-222'') – bisdisulfide dimer

US brand name: Opdivo™ (developed by Bristol-Myers Squibb, Medarex (Bristol-Myers Squibb) and Ono Pharmaceuticals)

167 clinical trials

2015 FDA Approved - Lung cancer (non-small cell)

2012 Start of Phase 3 - Lung cancer (non-small cell)

2011 Start of Phase 2 - Renal cell carcinoma

2006 Start of Phase 1 - Lung cancer (non-small cell)

Conditions: B-cell malignancies, Lung cancer (non-small cell), Melanoma, Renal Cell Carcinoma

Code name: BMS-936558, MDX-1106, ONO-4538, 5C4

CAS Registry number: 946414-94-4

Nivolumab is a fully human immunoglobulin (Ig) G4 monoclonal antibody directed against the negative immuno-regulatory human cell surface receptor programmed death-1 (PD-1, PCD-1). Nivolumab can bind to and block the activation of PD-1 by its ligands programmed cell death ligand 1 (PD-L1), overexpressed on certain cancer cells, and programmed cell death ligand 2 (PD-L2), which is primarily expressed on APCs. This can result in the activation of T-cells and cell-mediated immune responses against tumor cells or pathogens.

Compositions and methods of using and making nivolumab are disclosed, for example, in U.S. Patent Nos. 9,387,247; 8,779,105; 8,779,105; 8,168,179; and 8,008,449, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of nivolumab. In certain embodiments, the method is effective at sensitizing tumor cells to nivolomab. In certain embodiments, the method changes (e.g., enhances) the efficacy of nivolomab. In certain embodiments, the tumor cells are resistant to treatment with nivolomab alone.

Pembrolizumab

Synonym:	anti-PD-1 monoclonal antibody MK-3475
Description:	humanized mouse monoclonal (228-L-proline(H10->P)) gamma 4 heavy chain (134-218)-disulfide with humanized mouse monoclonal kappa light chain dimer (226-226":229-229")-bisdisulfide immunoglobulin G4, anti-(human programmed cell death 1)
US brand name:	Keytruda™ (developed by Merck and Schering-Plough);
	261 clinical trials
	2014 FDA Approved - Solid tumors
	2013 Start of Phase 3 - Melanoma
Code name:	MK-3475, SCH 900475, lambrolizumab
Conditions:	Melanoma, solid tumors

CAS Registry number: 1374853-91-4

Pembrolizumab is a humanized monoclonal immunoglobulin (Ig) G4 antibody directed against human cell surface receptor PD-1 (programmed death-1 or programmed cell death-1). Upon administration, pembrolizumab can bind to PD-1, an inhibitory signaling receptor expressed on the surface of activated T cells, and block the binding to and activation of PD-1 by its ligands, which can result in the activation of T-cell-mediated immune responses against tumor cells.

Compositions and methods of using and making pembrolizumab are disclosed, for example, in U.S. Patent Nos. 8,354,509; 8,900,587; 8,952,136; and 9,220,776, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of pembrolizumab. In certain embodiments, the method is effective at sensitizing tumor cells to pembrolizumab. In certain embodiments, the method changes (e.g., enhances) the efficacy of pembrolizumab. In certain embodiments, the tumor cells are resistant to treatment with pembrolizumab alone.

JS001

Synonym:	anti-PD-1 humanized antibody (developed by Shanghai Junshi Biosciences Inc. and Junmeng Biosciences Co., Ltd.)
Description:	Immunoglobulin G4, anti-(human programmed cell death protein 1) (human monoclonal JS001 γ4-chain), disulfide with human monoclonal JS001 κ-chain, dimer
Status:	2016 Start of Phase I clinical trials (NCT02836834, NCT02838823 NCT02836795 & NCT02857166).
Conditions:	Breast cancer, solid tumors

CAS Registry number: 1924598-82-2

Compositions and methods of using and making JS001 are disclosed, for example, in International Patent Application No. PCT/CN2014/072574, the content of which is hereby incorporated by reference herein in its entirety for any purpose

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor in a subject comprising administering to the subject an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of

JS001. In certain embodiments, the method is effective at sensitizing tumor cells to JS001. In certain embodiments, the method changes (e.g., enhances) the efficacy of JS001. In certain embodiments, the tumor cells are resistant to treatment with JS001 alone.

REGN2810

Synonym: anti-PD-1 human monoclonal antibody (developed by Regeneron Pharmaceuticals, Inc. and Sanofi-Aventis)

Description: Immunoglobulin G4, anti-(human programmed cell death protein 1) (human monoclonal REGN2810 heavy chain), disulfide with human monoclonal REGN2810 κ-chain, dimer

Status: 2015-2016 Start of Phase I/II clinical trials for treatment of advanced cutaneous squamous cell carcinoma (NCT02760498, NCT02651662, NCT02520245 & NCT02383212; see also J Clin Oncol 34, 2016 (suppl; abstr 3024)

Conditions: Advanced malignancies

CAS Registry number: 1801342-60-8

REGN2810 is a human monoclonal antibody directed against the negative immunoregulatory human cell surface receptor programmed cell death 1 (PD-1) protein. Upon administration, anti-PD-1 monoclonal antibody REGN2810 can bind to PD-1, inhibit its binding to the PD-1 ligand programmed cell death-1 ligand 1 (PD-L1), and prevent the activation of its downstream signaling pathways.

Compositions and methods of using and making REGN2810 are disclosed, for example, in the published U.S. Patent Application No. 2015/0203579, the content of which is hereby incorporated by reference herein in its entirety for any purpose

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of REGN2810. In certain embodiments, the method is effective at sensitizing tumor cells to REGN2810. In certain

embodiments, the method changes (e.g., enhances) the efficacy of REGN2810. In certain embodiments, the tumor cells are resistant to treatment with REGN2810 alone.

SHR-1210

Synonym: Immunoglobulin G4, anti-(cell surface receptor) (human-*Mus musculus* monoclonal SHR-1210 heavy chain), disulfide with human-*Mus musculus* monoclonal SHR-1210 light chain, dimer (developed by Incyte Corporation, Jiangsu Hengrui Medicine Co., Ltd and Shanghai Hengrui Pharmaceutical Company, Ltd.)

Description: Immunoglobulin G4-kappa, anti-[Homo sapiens PDCD1 (programmed cell death 1, PD-1, CD279)], humanized monoclonal antibody; gamma4 heavy chain (1-443) [humanized VH (Homo sapiens IGHV3-7*01 (90.80%) -(IGHD) -IGHJ4*01) [8.8.9] (1-116) -IGHG4*01 (CH1 (117-214), hinge S10>P (224) (215-226), CH2 (227-336), CH3 (337-441), CHS (442- 443)) (117-443)], (130-214') disulfide with kappa light chain (1'-214') [humanized V-KAPPA (Homo sapiens IGKV1-39*01 (87.40%) -IGKJ1*01) [6.3.9] (1'-107') -Homo sapiens IGKC*01, Km3 (108'-214')]; dimer (222-222":225 225")-bisdisulfide

Status: 2015-2016 Start of Phase I clinical trials for treatment of advanced melanoma and Advanced Solid Tumors (NCT02492789, NCT02721589, NCT02738489 & NCT02742935).

Conditions: Solid tumors (e.g., breast cancer, lung cancer)

Code name: INCSHR-1210; camrelizumab

CAS Registry Number: 1798286-48-2 (camrelizumab); 1923896-09-6 (SHR-1210)

SHR-1210 is a monoclonal antibody directed against the negative immunoregulatory human cell surface receptor programmed death-1 (PD-1). Upon administration, anti-PD-1 monoclonal antibody SHR-1210 can bind to and block the binding of PD-1 to its ligands programmed cell death ligand 1 (PD-L1), overexpressed on certain cancer cells, and programmed cell death ligand 2 (PD-L2), which is primarily expressed on antigen presenting cells (APCs).

Compositions and methods of using and making SHR-1210 are disclosed, for example, in International Patent Application No. PCT/CN2014/091090, the content of which is hereby incorporated by reference herein in its entirety for any purpose

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of SHR-1210. In certain embodiments, the method is effective at sensitizing tumor cells to SHR-1210. In certain embodiments, the method changes (e.g., enhances) the efficacy of SHR-1210. In certain embodiments, the tumor cells are resistant to treatment with SHR-1210 alone.

MEDI0680

Synonym:	anti-PD-1 human G4 monoclonal antibody (developed by Amplimmune Inc. & Medimmune)
Description:	Immunoglobulin G4, anti-(human programmed cell death 1 ligand protein PDCD1) (human-*Mus musculus* monoclonal MEDI0680 γ4-chain), disulfide with human-*Mus musculus* monoclonal MEDI0680 κ-chain, dimer
Status	2014 NCT02271945 (Phase I/Phase II) 2014 NCT02118337 (Phase I) 2013 NCT02013804 (Phase I)
Code name:	AMP-514, MEDI0680
Conditions:	Advanced malignancies, aggressive B cell lymphomas

CAS Registry Number: 1607465-69-9

MED10680 is a humanized immunoglobulin (Ig) G4 monoclonal antibody directed against the negative immunoregulatory human cell surface receptor programmed cell death 1 (PD-1. Upon administration, anti-PD-1 monoclonal antibody MEDI0680 can bind to and inhibit PD-1 and its downstream signaling pathways.

Compositions and methods of using and making MED10680 are disclosed, for example, in U.S. Patent No. 9,205,148 and the published U.S. Patent Application No. 2016/0130348, the

contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of MED10680. In certain embodiments, the method is effective at sensitizing tumor cells to MED10680. In certain embodiments, the method changes (e.g., enhances) the efficacy of MED10680. In certain embodiments, the tumor cells are resistant to treatment with MED10680 alone.

PDR001

Synonym:	humanized anti-PD-1 IgG4 antibody (developed by Novartis)
Status	2015-2016 Phase I/II clinical trials (NCT02807844, NCT02460224, NCT02404441, see also J Clin Oncol 34, 2016 (suppl; abstr 3060)
Conditions:	Advanced malignancies, solid tumors

CAS Registry Number: 1859072-53-9

PDR001 is a fully humanized monoclonal antibody that binds to PD-1 with high affinity and inhibits the biological activity of PD-1. Upon administration, anti-PD-1 monoclonal antibody PDR001 can bind to PD-1 expressed on activated T-cells and block the interaction with its ligands, programmed cell death 1 ligand 1 (PD-L1, PD-1L1) and PD-1 ligand 2 (PD-L2, PD-1L2).

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of PDR001. In certain embodiments, the method is effective at sensitizing tumor cells to PDR001. In certain embodiments, the method changes (e.g., enhances) the efficacy of PDR001. In certain embodiments, the tumor cells are resistant to treatment with PDR001 alone.

BGB-317

Synonym:	humanized anti-PD-1 monoclonal antibody (developed by BeiGene Ltd.)
Status	2015-2016 Phase I clinical trials (NCT02660034, NCT02407990; see also J Clin Oncol 34, 2016 (suppl; abstr 3066)
Code name:	BeiGene patent anti-PD-1
Conditions:	Advanced solid tumors

CAS Registry Number: 1863119-16-7

BGB-317 is a monoclonal antibody directed against the negative immunoregulatory human cell surface receptor programmed cell death 1 (PD-1). Upon administration, anti-PD-1 monoclonal antibody BGB-317 can bind to PD-1 and inhibit the binding of PD-1 to the PD-1 ligands programmed cell death-1 ligand 1 (PD-L1), and PD-1 ligand 2 (PD-L2).

Compositions and methods of using and making BGB-317 are disclosed, for example, in U.S. Patent Nos. 9,217,034 and 8,735,553, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of BGB-317. In certain embodiments, the method is effective at sensitizing tumor cells to BGB-317. In certain embodiments, the method changes (e.g., enhances) the efficacy of BGB-317. In certain embodiments, the tumor cells are resistant to treatment with BGB-317 alone.

TSR-042

Synonym:	humanized anti-PD-1 monoclonal antibody (developed by AnaptysBio and Tesaro, Inc.)
Status	2016 Phase I clinical trial (NCT02715284)
Code name:	ANB011, TSR-042
Conditions:	Advanced solid tumors

CAS Registry Number: 1923896-12-1

TSR-042 is a humanized monoclonal antibody directed against the negative immunoregulatory human cell surface receptor programmed cell death 1 (PD-1; programmed death-1), with potential immune checkpoint inhibitory and antineoplastic activities. Upon administration, anti-PD-1 monoclonal antibody TSR-042 can bind to and inhibit PD-1 and its downstream signaling pathways.

Compositions and methods of using and making TSR-042 are disclosed, for example, in U.S. published Patent Application Patent No. 2016/0075783, the content of which is hereby incorporated by reference herein in its entirety for any purpose

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of TSR-042. In certain embodiments, the method is effective at sensitizing tumor cells to TSR-042. In certain embodiments, the method changes (e.g., enhances) the efficacy of TSR-042. In certain embodiments, the tumor cells are resistant to treatment with TSR-042 alone.

PF-06801591

Synonym:	Anti-PD-1 monoclonal antibody
Status:	2016 NCT02573259 Phase 1 (developed by Pfizer & Merck)
Conditions:	Melanoma, Head and Neck Cancer (SCHNC), Ovarian, Sarcoma, Hodgkin Lymphoma

PF-06801591 is an inhibitor of the human inhibitory receptor programmed cell death 1 (PD-1; PDCD1), with potential immune checkpoint inhibitory and antineoplastic activities. Upon administration, anti-PD-1 checkpoint inhibitor PF-06801591 can target and bind to PD-1 and blocks the interaction between PD-1 and its ligands, PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2).

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor

comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of PF-06801591. In certain embodiments, the method is effective at sensitizing tumor cells to PF-06801591. In certain embodiments, the method changes (e.g., enhances) the efficacy of PF-06801591. In certain embodiments, the tumor cells are resistant to treatment with PF-06801591 alone.

Ningbo Cancer Hosp. (NCH) anti-PD-1 CAR

Synonym:	Anti-PD-1 Chimeric antigen receptor
Status:	2016 NCT02873390 Phase 1/II
	(developed by Ningbo Cancer Hospital, China)
Conditions:	EGFR Family Member Positive Advanced Malignancies
Code name:	HerinCAR-PD-1 cells (PD-1 Antibody Expressing CAR-T Cells)

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of NCH anti-PD-1 CAR. In certain embodiments, the method is effective at sensitizing tumor cells to NCH anti-PD-1 CAR. In certain embodiments, the method changes (e.g., enhances) the efficacy of NCH anti-PD-1 CAR. In certain embodiments, the tumor cells are resistant to treatment with NCH anti-PD-1 CAR alone.

Pidilizumab

Synonyms:	CT-011, MK-3475, hBat-1, MDV9300
Status:	NCT02077959 Phase I/II: Lenalidomide and pidilizumab are currently being evaluated for the treatment of patients with relapsed or refractory multiple myeloma (developed by CureTech Ltd. Medivation Inc., Teva Pharmaceutical Industries)
Description:	Immunoglobulin G1, anti-(receptor PD-1 (Programmed Death 1)) (human-mouse monoclonal CT-011 γ1-chain), disulfide with human-mouse monoclonal CT-011 κ-chain, dimer

CAS Registry Number: 1036730-42-3

Compositions and methods of using and making pidilizumab are disclosed, for example, in U.S. Patent Nos. US7332582; US8686119; US8747847; US9309308 and US9416175, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of pidilizumab. In certain embodiments, the method is effective at sensitizing tumor cells to pidilizumab. In certain embodiments, the method changes (e.g., enhances) the efficacy of pidilizumab. In certain embodiments, the tumor cells are resistant to treatment with pidilizumab alone.

TABLE 7 provides non-limiting examples of pre-clinical inhibitors of the PD-1 immune checkpoint reported in the recited "Related Patent Documents," each one of which is hereby incorporated by reference herein in its entirety for any purpose

TABLE 7

Preclinical Inhibitors of PD-1	Company	Related Patent Documents
Medimmune anti-PD-1	Medimmune, Wyeth	Compositions and methods of using and making Medimmune anti-PD-1 are disclosed in US7488802; US7029674; US20160222113, the contents of which are hereby incorporated by reference herein in their entireties for any purpose
Isis anti-PD-1	Isis	Compositions and methods of using and making Isis anti-PD-1 are disclosed in WO2010029434; WO2010029435; US20110171220; US20110171215; US8927697, the contents of which are hereby incorporated by reference herein in their entireties for any purpose
UCB anti-PD-1 or 948.g1	UCB	Compositions and methods of using and making UCB anti-PD-1 are disclosed in US20160068586; US9102728; US8993731, the contents of which are

Preclinical Inhibitors of PD-1	Company	Related Patent Documents
		hereby incorporated by reference herein in their entireties for any purpose
Dana-Farber anti-PD-1	Dana-Farber, Novartis	Compositions and methods of using and making Dana-Farber anti-PD-1 are disclosed in US9102727; US20160137731; US20110271358; US20130291136; WO2015112900; WO2010036959, the contents of which are hereby incorporated by reference herein in their entireties for any purpose
STI-1110	Sorrento	Compositions and methods of using and making STI-1110 are disclosed in US20140356363; WO2014194302, the contents of which are hereby incorporated by reference herein in their entireties for any purpose
Suzhou Stainwei Biotech anti-PD-1	Suzhou Stainwei Bio	Compositions and methods of using and making Suzhou Stainwei Biotech anti-PD-1 are disclosed in WO2015058573, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Haixi pembrolizumab biosimilar	Haixi	
Livzon anti-PD-1	Livzon	Compositions and methods of using and making Livzon anti-PD-1 are disclosed in WO2016015685, the content of which is hereby incorporated by reference herein in its entirety for any purpose
MabQuest anti-PD-1	MabQuest	Compositions and methods of using and making MabQuest anti-PD-1 are disclosed in WO2016020856, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Singapore ASTR anti-PD-1	Singapore ASTR	Compositions and methods of using and making Singapore ASTR anti-PD-1 are disclosed in WO2016068801, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Sutro anti-PD-1	Sutro	Compositions and methods of using and making Sutro anti-PD-1 are disclosed in WO2016077397, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Rinat anti-PD-1	Rinat	Compositions and methods of using and making Rinat anti-PD-1 are disclosed in US20160159905; WO2016092419, the contents of which are hereby

Preclinical Inhibitors of PD-1	Company	Related Patent Documents
		incorporated by reference herein in their entireties for any purpose
Biocad anti-PD-1	Biocad	
Enumeral anti-PD-1 or ENUM 388D4	Enumeral	Compositions and methods of using and making ENUM 388D4 anti-PD-1 are disclosed in WO2016106159, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Kadmon anti-PD-1	Kadmon	Compositions and methods of using and making Kadmon anti-PD-1 are disclosed in WO2016127179, the content of which is hereby incorporated by reference herein in its entirety for any purpose

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of a preclinical inhibitor of PD1 shown in TABLE 7. In certain embodiments, the method is effective at sensitizing tumor cells to the preclinical inhibitor of PD1. In certain embodiments, the method changes (e.g., enhances) the efficacy of the preclinical inhibitor of PD1. In certain embodiments, the tumor cells are resistant to treatment with the preclinical inhibitor of PD1 alone.

Exemplary PD-L1 immune checkpoint inhibitors that may be combined with a modulator of KRAS signaling, as disclosed herein, include, but are not limited, to:

Atezolizumab

Synonym: Anti-PD-L1 monoclonal antibody MPDL3280A

Description: Immunoglobulin G1, anti-(human CD antigen CD274) (human monoclonal MPDL3280A heavy chain), disulfide with human monoclonal MPDL3280A κ-chain, dimer

US brand name: Tecentriq™ (developed by Genentech/Hoffmann-La Roche)

FDA approved for the treatment of bladder cancer

Code name: MPDL3280A, RG7446, RO5541267, YW243.55.S70

CAS Registry Number: 1380723-44-3

Atezolizumab is a human, Fc optimized, monoclonal antibody directed against the protein ligand PD-L1 (programmed cell death-1 ligand 1), with potential immune checkpoint inhibitory and antineoplastic activities. PD-L1 is overexpressed on many human cancer cell types and on various tumor-infiltrating immune cells. Atezolizumab can bind to PD-L1, blocking its binding to and activation of its receptor programmed death 1 (PD-1) expressed on activated T-cells, which may enhance the T-cell-mediated immune response to neoplasms and reverse T-cell inactivation. In addition, by binding to PD-L1, atezolizumab can also prevent binding of this ligand to B7.1 expressed on activated T cells, which further enhances the T-cell-mediated immune response. The Fc region of atezolizumab is modified in such a way that it does not induce either antibody-dependent cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).

Compositions and methods of using and making atezolizumab are disclosed, for example, in U.S. Patent No. 8,217,149, the content of which is hereby incorporated by reference herein in its entirety for any purpose

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of atezolizumab. In certain embodiments, the method is effective at sensitizing tumor cells to atezolizumab. In certain embodiments, the method changes (e.g., enhances) the efficacy of atezolizumab. In certain embodiments, the tumor cells are resistant to treatment with atezolizumab alone.

Avelumab

Synonym: Anti-PD-L1 monoclonal antibody

Description: Immunoglobulin G1, anti-(human CD antigen CD274) (human monoclonal MSB0010718C heavy chain), disulfide with human monoclonal MSB0010718C light chain, dimer

Code name: MSB0010718C, A09-246-2 (developed by Merck Serono and Pfizer)
CAS Registry Number: 1537032-82-8

Avelumab is a human immunoglobulin G1 (IgG1) monoclonal antibody directed against the human immunosuppressive ligand programmed death-ligand 1 (PD-L1) protein, with potential immune checkpoint inhibitory and antineoplastic activities. Upon administration, avelumab can bind to PD-L1 and prevent the interaction of PD-L1 with its receptor programmed cell death protein 1 (PD-1). This can inhibit the activation of PD-1 and its downstream signaling pathways. This may restore immune function through the activation of cytotoxic T-lymphocytes (CTLs) targeted to PD-L1-overexpressing tumor cells. In addition, avelumab can induce an antibody-dependent cellular cytotoxic (ADCC) response against PD-L1-expressing tumor cells.

Compositions and methods of using and making avelumab are disclosed, for example, in the published International Patent Publication Nos. WO2013079174 and WO2016137985, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of avelumab. In certain embodiments, the method is effective at sensitizing tumor cells to avelumab. In certain embodiments, the method changes (e.g., enhances) the efficacy of avelumab. In certain embodiments, the tumor cells are resistant to treatment with avelumab alone.

Durvalumab

Code name: MEDI-4736, MEDI4736
(Developed by Medimmune/AstraZeneca/Celgene)
Description: Immunoglobulin G1, anti-(human protein B7-H1) (human monoclonal MEDI4736 heavy chain), disulfide with human monoclonal MEDI4736 kappa-chain, dimer
CAS Registry Number: 1428935-60-7

Durvalumab is an Fc optimized monoclonal antibody directed against programmed cell death-1 ligand 1 (PD-L1; B7 homolog 1; B7H1), with potential immune checkpoint inhibitory and antineoplastic activities. Upon intravenous administration, durvalumab can bind to PD-L1, thereby blocking its binding to and activation of its receptor programmed death 1 (PD-1) expressed on activated T-cells. This may reverse T-cell inactivation and activate the immune system to exert a cytotoxic T-lymphocyte (CTL) response against PD-L1-expressing tumor cells. The Fc region of durvalumab is modified in such a way that it does not induce either antibody-dependent cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).

Compositions and methods of making and using durvalumab are disclosed, for example, in U.S. Patent No. 8,779,108 and the published U.S. Patent Application No. 2016/0222120, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of durvalumab. In certain embodiments, the method is effective at sensitizing tumor cells to durvalumab. In certain embodiments, the method changes (e.g., enhances) the efficacy of durvalumab. In certain embodiments, the tumor cells are resistant to treatment with durvalumab alone.

AMP-224

Code name:	B7-DC Ig (Developed by Medimmune/Amplimmune/ AstraZeneca)
Description:	AMP-224 is a recombinant B7-DC Fc-fusion protein composed of the extracellular domain of the PD-1 ligand programmed cell death ligand 2 (PD-L2, B7-DC) and the Fc region of human immunoglobulin (Ig) G1

CAS Registry Number: 1422184-00-6

AMP-224 is a recombinant B7-DC Fc-fusion protein that binds to PD-1. In a mouse model of colon cancer, an optimized treatment regimen with AMP-224 eradicated established tumors of the animals and conferred long-term protective anti-cancer immunity.

Compositions and methods of making and using AMP-224 are disclosed, for example, in the International Publication Nos. WO2010027827 and WO2011066342, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of AMP-224. In certain embodiments, the method is effective at sensitizing tumor cells to AMP-224. In certain embodiments, the method changes (e.g., enhances) the efficacy of AMP-224. In certain embodiments, the tumor cells are resistant to treatment with AMP-224.

MDX-1105

Code name: MDX-1105, BMS-936559, 12A4
(Developed by Medarex, (Bristol-Myers Squibb)
anti-PD-L1 antibody

CAS Registry Number: 1422185-22-5

MDX-1105 is an anti-PD-L1 antibody that is in Phase I/II clinical trials for the treatment of advanced cancers, including hematologic malignancies, melanoma, renal cell carcinoma and solid tumors (Brahmer *et al.* N. Engl. J. Med. (2012) 366:2455-65).

Compositions and methods of making and using AMP-224 are disclosed, for example, in U.S. Patent Nos. 7,943,743; 8,383,796; 9,102,725; 9,212,224 and 9,273,135, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS

aiRNA, as defined herein, combined with an effective amount of MDX-1105. In certain embodiments, the method is effective at sensitizing tumor cells to MDX-1105. In certain embodiments, the method changes (e.g., enhances) the efficacy of MDX-1105. In certain embodiments, the tumor cells are resistant to treatment with MDX-1105.

LY3300054

LY3300054 is an anti-PD-L1 antibody that has entered Phase I clinical trial in June 2016 for the treatment of advanced refractory solid tumors (Clinical Trial ID: NCT02791334)

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of LY3300054. In certain embodiments, the method is effective at sensitizing tumor cells to LY3300054. In certain embodiments, the method changes (e.g., enhances) the efficacy of LY3300054. In certain embodiments, the tumor cells are resistant to treatment with LY3300054.

KN035

Alphamab is an anti-PD-L1 heavy chain antibody selected from a large camel naïve phage display Nanobody library.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of Alphamab. In certain embodiments, the method is effective at sensitizing tumor cells to Alphamab. In certain embodiments, the method changes (e.g., enhances) the efficacy of Alphamab. In certain embodiments, the tumor cells are resistant to treatment with Alphamab.

TABLE 7 provides non-limiting examples of pre-clinical inhibitors of the PD-L1 immune checkpoint reported in the recited "Related Patent Documents," each one of which is hereby incorporated by reference herein in its entirety for any purpose

TABLE 8

Preclinical Inhibitors of PD-L1	Company	Related Patent Documents/ Scientific Literature
Mayo patent anti-B7-H1	Mayo	Compositions and methods of using and making Mayo patent anti-B7-H1 are disclosed in US8460927; US8981063, the contents of which are hereby incorporated by reference herein in their entireties for any purpose
Dana-Farber patent anti-PD-L1	Checkpoint Therapeutics Dana-Farber Novartis	Compositions and methods of using and making Dana-Farber patent anti-PD-L1 are disclosed in US20160108123; WO2016061142; US20160137731; WO2015061668; US20150274835; WO2014055897; WO2014022758; US20150197571; US20130291136; US8552154; WO2010036959; US20110271358, the contents of which are hereby incorporated by reference herein in their entireties for any purpose
STI-1014	Sorrento	Compositions and methods of using and making STI-1014 are disclosed in US9175082, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Regeneron patent anti-PD-L1	Regeneron	Compositions and methods of using and making Regeneron patent anti-PD-L1 are disclosed in US20150203580; WO2015112805, the contents of which are hereby incorporated by reference herein in their entireties for any purpose
Kadmon patent anti-PD-L1	Kadmon	Compositions and methods of using and making Kadmon patent anti-PD-L1 are disclosed in WO2015109124, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Merck patent anti-PD-L1 / TGF beta RII	Merck Serono	Compositions and methods of using and making Merck patent

Preclinical Inhibitors of PD-L1	Company	Related Patent Documents/ Scientific Literature
		anti-PD-L1 / TGF beta RII are disclosed in WO2015118175, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Merck patent anti-PD-L1	Merck & Co	Compositions and methods of using and making Merck patent anti-PD-L1 are disclosed in WO2014100079, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Spring Bioscience patent anti-PD-L1	Genentech Spring Bioscience	Compositions and methods of using and making Spring Bioscience patent anti-PD-L1 are disclosed in WO2015181342; WO2016007235, the contents of which are hereby incorporated by reference herein in their entireties for any purpose
R-Pharm patent anti-PD-L1	R-Pharm	Compositions and methods of using and making R-Pharm patent anti-PD-L1 are disclosed in WO2015195163, the content of which is hereby incorporated by reference herein in its entirety for any purpose
BeiGene patent anti-PD-L1	BeiGene	Compositions and methods of using and making BeiGene patent anti-PD-L1 are disclosed in WO2016000619, the content of which is hereby incorporated by reference herein in its entirety for any purpose
B60-55	DingFu Biotarget	Compositions and methods of using and making B60-55 are disclosed in Luan *et al.* Int. Immunopharmacol. (2016) 31:248-256, the content of which is hereby incorporated by reference herein in its entirety for any purpose

Preclinical Inhibitors of PD-L1	Company	Related Patent Documents/ Scientific Literature
Zha *et al.* patent anti-PD-L1		Compositions and methods of using and making Zha *et al.* patent anti-PD-L1 are disclosed in WO2016022630, (the content of which is hereby incorporated by reference herein in its entirety for any purpose
Roche patent anti-PD-L1	Roche	Compositions and methods of using and making Roche patent anti-PD-L1 are disclosed in WO2016030350, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Kadmon anti-VEGFR2 / PD-L1	Kadmon	AACR 2016; Abstract ID: 572 A novel anti-PDL1 x anti-VEGFR2 bispecific antibody for enhanced antitumor immunity, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Singapore ASTR patent anti-PD-L1	Singapore ASTR	Compositions and methods of using and making Singapore ASTR patent anti-PD-L1 are disclosed in WO2016111645, the content of which is hereby incorporated by reference herein in its entirety for any purpose

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of a preclinical inhibitor of PD-L1 shown in TABLE 7. In certain embodiments, the method is effective at sensitizing tumor cells to the preclinical inhibitor of PD-L1. In certain embodiments, the method changes (e.g., enhances) the efficacy of the preclinical inhibitor of PD-L1. In certain embodiments, the tumor cells are resistant to treatment with the preclinical inhibitor of PD-L1 alone.

Exemplary PD-L2 immune checkpoint inhibitors that may be combined with a modulator of KRAS signaling, as disclosed herein, include, but are not limited, to:

rHIgM12B7

Status: Currently being evaluated in a Phase I clinical trial NCT00658892 for the treatment of Stage IV Melanoma.

Synonym: Mayo patent anti-PD-L2

A recombinant form of the monoclonal IgM antibody M12 isolated from a Waldenstrom macroglobulinaemia patient (rHIgM12) with potential immunomodulating activity. B7-DC cross-linking antibody rHIgM12B7 binds and crosslinks the B7 co-stimulatory family member B7-DC (PD-L2) on dendritic cells (DCs), antigen presenting cells (APCs) that play a crucial role in the human immune response.

Compositions and methods of using and making rHIgM12B7 are disclosed in the published U.S. Patent Application No. 2016/0122431 and U.S. Patent No. 9,255,147, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of rHIgM12B7. In certain embodiments, the method is effective at sensitizing tumor cells to rHIgM12B7. In certain embodiments, the method changes (e.g., enhances) the efficacy of rHIgM12B7. In certain embodiments, the tumor cells are resistant to treatment with rHIgM12B7 alone

Dana-Farber patent anti-PD-L2 (preclinical)

Compositions and methods of using and making the Dana-Farber patent anti-PD-L2 are disclosed in the published U.S. Patent Application Nos. 2011/0271358; 2013/0291136; 2015/0197571 and 2016/0137731, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

Exemplary CTLA-4 immune checkpoint inhibitors that may be combined with a modulator of KRAS signaling, as disclosed herein, include, but are not limited, to:

Ipilimumab

Synonym:	anti-cytotoxic T-lymphocyte-associated antigen-4 monoclonal antibody; Monoclonal antibody CTLA-4 (MOAB CTLA-4)
US brand name:	Yervoy™ (developed by Bristol-Myers Squibb) FDA approved for the treatment of melanoma
Code name:	BMS-734016, MDX-010, MDX101, MDX-CTLA-4, 10D1
Description:	immunoglobulin G1, anti-(human CTLA-4 (antigen)) (human γ1-chain), disulfide with human κ-chain, dimer

CAS Registry Number: 477202-00-9

Ipilimumab is a recombinant human immunoglobulin (Ig) G1 monoclonal antibody directed against the human T-cell receptor cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), with immune checkpoint inhibitory and antineoplastic activities. Ipilimumab can bind to CTLA4 expressed on T-cells and inhibit the CTLA4-mediated downregulation of T-cell activation. This can lead to a cytotoxic T-lymphocyte (CTL)-mediated immune response against cancer cells.

Compositions and methods of making and using ipilimumab are disclosed, for example, in the U.S. Patent Nos. 9,358,289; 9,320,811; 8,784,815; 9,062,111; 8,685,394; 8,475,790; 8,119,129; 8,449,886; 8,110,194; 6,984,720 and the International Publication Nos. WO2015134605, WO2014066532, WO2013142796, WO2013019620, WO2012027536, WO2011045704, WO2011011027, WO2010042433, WO2009100140, WO2007067959, WO2007056539, WO2003086459, WO2001014424, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of ipilimumab. In certain embodiments, the method is effective at sensitizing tumor cells to ipilimumab. In certain

embodiments, the method changes (e.g., enhances) the efficacy of ipilimumab. In certain embodiments, the tumor cells are resistant to treatment with ipilimumab alone.

Tremelimumab

Synonym: anti-CTLA4 human monoclonal antibody CP-675,206; ticilimumab

Description: immunoglobulin G2, anti-(human CTLA-4 (antigen) (human monoclonal CP-675206 clone 11.2.1 heavy chain) disulfide with human monoclonal CP-675206 clone 11.2.1 light chain, dimer

Code name: CP-675; CP-675, 206 (developed by AstraZeneca)

CAS Registry Number: 477202-00-9

Tremelimumab is a human immunoglobulin (Ig) G2 monoclonal antibody directed against the human T-cell receptor protein cytotoxic T-lymphocyte-associated protein 4 (CTLA4), with potential immune checkpoint inhibitory and antineoplastic activities. Tremelimumab can bind to CTLA4 on activated T-lymphocytes and block the binding of the antigen-presenting cell ligands B7-1 (CD80) and B7-2 (CD86) to CTLA4, resulting in inhibition of CTLA4-mediated downregulation of T-cell activation. This can promote the interaction of B7-1 and B7-2 with another T-cell surface receptor protein CD28, and result in a B7-CD28-mediated T-cell activation that is unopposed by CTLA4-mediated inhibition.

Compositions and methods of making and using tremelimumab are disclosed, for example, in U.S. Patent Nos. 8,883,984; 8,491,895; 8,685,394; 7,824,679; 8,143,379; 7,411,057; 7,132,281; 7,109,003; 6,682,736 and the International Publication Nos. WO2016030455, WO2015173267, WO2011045704, WO2007113648, WO2006101691, WO2006101692, WO2006096491, WO2006048749, WO2005092380, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of tremelimumab. In certain embodiments, the method is effective at sensitizing tumor cells to tremelimumab. In certain

embodiments, the method changes (e.g., enhances) the efficacy of tremelimumab. In certain embodiments, the tumor cells are resistant to treatment with tremelimumab alone.

AGEN1884

AGEN-1884 developed by Agenus is an anti-CTLA-4 antibody that is Phase I clinical trials for the treatment of solid tumors (Clinical Trial ID: NCT02694822).

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of AGEN-1884. In certain embodiments, the method is effective at sensitizing tumor cells to AGEN-1884. In certain embodiments, the method changes (e.g., enhances) the efficacy of AGEN-1884. In certain embodiments, the tumor cells are resistant to treatment with AGEN-1884 alone.

TABLE 8 provides non-limiting examples of pre-clinical inhibitors of the CTLA-4 immune checkpoint reported in the recited "Related Patent Documents," each one of which is hereby incorporated by reference herein in its entirety for any purpose

TABLE 9

Preclinical Inhibitors of PD-L1	Company	Related Patent Documents/ Scientific literature
Tegenero patent anti-CTLA4 (TGN2122, TGN2422)	Tegenero	Compositions and methods of using and making Tegenero patent anti-CTLA4 are disclosed in WO2006066568, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Genetics Inst. patent anti-CTLA4	Genetics Institute	Compositions and methods of using and making Genetics Inst. patent anti-CTLA4 are disclosed in WO2001054732 (the content of which is hereby incorporated by reference herein in its entirety for any purpose)
Antitope patent anti-CTLA4 (VH5:VK4)	Antitope	Compositions and methods of using and making Antitope patent anti-CTLA4 are disclosed in WO2012120125, the content of which is hereby incorporated by reference herein in its entirety for any purpose

Preclinical Inhibitors of PD-L1	Company	Related Patent Documents/ Scientific literature
Taiwan patent anti-CTLA4	Undefined Taiwan	Compositions and methods of using and making Taiwan patent anti-CTLA4 are disclosed in US20130267688, the content of which is hereby incorporated by reference herein in its entirety for any purpose
JMW-3B3	U Aberdeen	Compositions and methods of using and making JMW-3B3 anti-CTLA4 are disclosed in US8697845, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Biomed Valley patent anti-CTLA-4 / PD-1	Biomed Valley	Compositions and methods of using and making Biomed Valley patent anti-CTLA-4 / PD-1 are disclosed in WO2014209804, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Alligator Bio patent anti-CTLA-4 / CD40	Alligator	Compositions and methods of using and making Alligator Bio patent anti-CTLA-4 / CD40 are disclosed in WO2014207064, the content of which is hereby incorporated by reference herein in its entirety for any purpose
Akeso patent anti-CTLA4	Akeso	Compositions and methods of using and making Akeso patent anti-CTLA4 are disclosed in WO2016015675, the content of which is hereby incorporated by reference herein in its entirety for any purpose
XmAb20717	Xencor	XmAb20717 is a bispecific PD-1 x CTLA-4 checkpoint inhibitor under evaluation for the treatment of neuroendocrine tumors and multiple cancers.
Sorrento patent anti-CTLA4	Sorrento	Compositions and methods of using and making Sorrento patent anti-CTLA4 are disclosed in WO2016130898 and WO2016130986, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.
ONC-392	OncoImmune, Pfizer	

Exemplary VISTA immune checkpoint inhibitors that may be combined with a modulator of KRAS signaling, as disclosed herein, include, but are not limited, to:

Janssen patent anti-VISTA antibody (preclinical)

Compositions and methods of making and using Janssen patent anti-VISTA antibody are disclosed, for example, in WO2015097536, the content of which is hereby incorporated by reference herein in its entirety.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of Janssen patent anti-VISTA antibody. In certain embodiments, the method is effective at sensitizing tumor cells to Janssen patent anti-VISTA antibody. In certain embodiments, the method changes (e.g., enhances) the efficacy of Janssen patent anti-VISTA antibody. In certain embodiments, the tumor cells are resistant to treatment with Janssen patent anti-VISTA antibody alone.

Igenica patent anti-C10orf54 antibody

Compositions and methods of making and using Igenica patent anti-C10orf54 antibody are disclosed, for example, in WO2014197849 and WO2016094837, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of Igenica patent anti-C10orf54 antibody. In certain embodiments, the method is effective at sensitizing tumor cells to Igenica patent anti-C10orf54. In certain embodiments, the method changes (e.g., enhances) the efficacy of Igenica patent anti-C10orf54 antibody. In certain embodiments, the tumor cells are resistant to treatment with Igenica patent anti-C10orf54 antibody alone.

Amplimmune patent anti-B7-H5 antibody

Compositions and methods of making and using Amplimmune patent anti-B7-H5 antibody are disclosed, for example, in WO2014190356 and the published U.S. Patent

Application No. 2016/0096891, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein. In certain embodiments, the present disclosure provides a method for enhancing an immune response against a tumor comprising administering an effective amount of a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, combined with an effective amount of Igenica patent Amplimmune patent anti-B7-H5 antibody. In certain embodiments, the method is effective at sensitizing tumor cells to Amplimmune patent anti-B7-H5 antibody. In certain embodiments, the method changes (e.g., enhances) the efficacy of Amplimmune patent anti-B7-H5 antibody. In certain embodiments, the tumor cells are resistant to treatment with Amplimmune patent anti-B7-H5 antibody alone.

In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of a co-administered therapeutic agent.

In certain embodiments, the change in the efficacy of a therapeutic agent. as a result of the co-administration of a modulator of KRAS signaling as defined herein, can be evaluated in subcutaneous tumor animal models at endpoints such as the percent test/control (%T/C) tumor weights calculated on each day that tumors are measured, tumor growth delay, net log cell kill, median days to a defined tumor weight or to a specified number of tumor doublings, and tumor regression. In certain embodiments, the lowest calculated %T/C seen over time can be defined as the optimal %T/C because it defines the greatest level of activity seen with the therapeutic agent. The rate and duration of partial and complete tumor regressions can also be considered to be clinically relevant endpoints.

For example, a T/C = 0% means no tumor growth. A T/C = 100% means no antitumor activity, i.e., the treated and control tumors grew equally. A T/C equal to or less than 42% is considered significant antitumor activity by the Drug Evaluation Branch of the Division of Cancer Treatment (NCI). A T/C value < 10% is considered to indicate highly significant antitumor activity, and is the level used by NCI to justify a clinical trial if toxicity, formulation, and certain other requirements are met (termed DN- 2 level activity).

In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of radiotherapy. In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with radiotherapy. In certain embodiments, the method is effective at enhancing the efficacy of the radiotherapy.

In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of an anti-angiogenesis agent. In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with an anti-angiogenesis agent. In certain embodiments, the method is effective at enhancing the efficacy of the anti-angiogenesis agent.

Non-limiting examples of anti-angiogenesis agents include, for example, MMP-2 (matrix-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloproteinase 9) inhibitors, and COX- 11 (cyclooxygenase 11) inhibitors, rapamycin, temsirolimus (CCI-779), everolimus (RAD001), sorafenib, sunitinib, and bevacizumab. COX-II inhibitors include but are not limited to, Celebrex™ (alecoxib), valdecoxib, and rofecoxib.

Examples of useful matrix metalloproteinase inhibitors are described in WO 96/33172, WO 96/27583, WO 98/07697, WO 98/03516, WO 98/34918, WO 98/34915, WO 98/33768, WO 98/30566, WO 90/05719, WO 99/52910, WO 99/52889, WO 99/29667, PCT International Application No. PCT/IB98/01113, U.S. Patent Nos. 5,863,949 and 5,861,510, all of which are incorporated by reference herein in their entireties for any purpose. Examples of MMP inhibitors include, but are not limited to, AG-3340, RO 32-3555, and RS 13-0830.

In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of a chemotherapeutic agent. In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with a chemotherapeutic agent. In certain embodiments, the chemotherapeutic agent includes, but not limited to, antimetabolites, antibiotics, alkylating agents, plant alkaloids, and/or hormonal agents. In certain embodiments, the method is effective at enhancing the efficacy of the chemotherapeutic agent.

Non-limiting examples of chemotherapeutic agents (e.g., suitable for use in compositions and methods described herein) include, but are not limited to:

- alkaloids, including microtubule inhibitors (e.g., vincristine, vinblastine, and vindesine, etc.), microtubule stabilizers (e.g., paclitaxel (Taxol), and docetaxel (taxotere), etc.), and chromatin function inhibitors, including topoisomerase inhibitors, such as epipodophyllotoxins (e.g., etoposide (VP-16), and teniposide (VM-26), etc.), and agents that target topoisomerase I (e.g., camptothecin and isirinotecan (CPT-11), etc.);
- covalent DNA-binding agents (alkylating agents), including nitrogen mustards (e.g., mechlorethamine, chlorambucil, cyclophosphamide, ifosphamide, and busulfan (Myleran), etc.), nitrosoureas (e.g., carmustine, lomustine, and semustine, etc.), and other alkylating agents (e.g., dacarbazine, hydroxymethylmelamine, thiotepa, and mitomycin, etc.);
- noncovalent DNA-binding agents (antitumor antibiotics), including nucleic acid inhibitors (e.g., dactinomycin (actinomycin D), etc.), anthracyclines (e.g., daunorubicin (daunomycin, and cerubidine), doxorubicin (adriamycin), and idarubicin (idamycin), etc.), anthracenediones (e.g., anthracycline analogues, such as mitoxantrone, etc.), bleomycins (Blenoxane), etc., and plicamycin (mithramycin), etc.;
- antimetabolites, including antifolates (e.g., methotrexate, Folex, and Mexate, etc.), purine antimetabolites (e.g., 6-mercaptopurine (6-MP, Purinethol), 6-thioguanine (6-TG), azathioprine, acyclovir, ganciclovir, chlorodeoxyadenosine, 2-chlorodeoxyadenosine (CdA), and 2'-deoxycoformycin (pentostatin), etc.), pyrimidine antagonists (e.g., fluoropyrimidines (e.g., 5-fluorouracil (Adrucil), 5-fluorodeoxyuridine (FdUrd) (floxuridine)) etc.), and cytosine arabinosides (e.g., Cytosar (ara-C) and fludarabine, etc.); 5) enzymes, including L-asparaginase, and hydroxyurea, etc.;
- hormones, including glucocorticoids, antiestrogens (e.g., tamoxifen, etc.), nonsteroidal antiandrogens (e.g., flutamide, etc.), and aromatase inhibitors (e.g., anastrozole (Arimidex), etc.);
- platinum compounds (e.g., cisplatin and carboplatin, etc.);

In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the

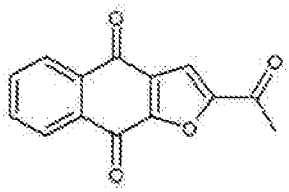
modulator of KRAS signaling changes (e.g., enhances) the efficacy of a cancer stemness inhibitor. In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, a cancer stemness inhibitor.

As used herein, the term "cancer stemness inhibitor" means a molecule that can target, reduce, inhibit, interfere with, or modulate at least one of a plurality of pathways involved in cancer stemness or the expression (e.g., the production of a functional product, e.g., a protein) of at least one of a plurality of cancer stemness genes.

In certain embodiments, a cancer stemness inhibitor can be, for example, a small molecule that selectively binds a protein encoded by a cancer stemness gene. In certain embodiments, a cancer stemness inhibitor is a biologic, e.g., a recombinant binding protein or peptide (e.g. APTSTAT3) or nucleic acid (e.g. STAT3 aiRNA; see U.S. Patent No. 9,328,345, the content of which is incorporated by reference herein in its entirety for any purpose), or conjugate thereof. In certain embodiments, a cancer stemness inhibitor is a cell. In certain embodiments, a cancer stemness inhibitor is a STAT3 inhibitor that binds to and inhibits a biological activity of STAT3. In certain embodiments, STAT3 refers to mammalian STAT3. In certain embodiments, STAT3 refers to the human "Signal Transducer and Activator of Transcription 3" having a canonical 770 amino acid sequence (Accession No: P40763-1; NP_644805.1). STAT3, as used herein, includes pSTAT3 or pSTAT3 dimer.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, a cancer stemness inhibitor. In certain embodiments, a cancer stemness inhibitor can be, for example, a compound chosen from 2-(1-hydroxyethyl)-naphtho[2,3-b]furan-4,9-dione, 2-acetyl-7-chloro-naphtho[2,3-b]furan-4,9-dione, 2-acetyl-7-fluoro-naphtho[2,3-b]furan-4,9-dione, 2-acetylnaphtho[2,3-b]furan-4,9-dione, or 2-ethyl-naphtho[2,3-b]furan-4,9-dione, prodrugs thereof, derivatives thereof, pharmaceutically acceptable salts of any of the foregoing, and solvates of any of the foregoing.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, a cancer stemness gene inhibitor chosen from compounds having formula A, shown below,



(A)

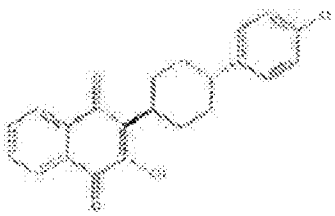
or prodrugs, derivatives, pharmaceutically acceptable salts or solvates thereof.

The compound having formula A may also be known as 2-acetylnaphtho[2,3-b]furan-4,9-dione, napabucasin, or BBI608 and include tautomers thereof. In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of 2-acetylnaphtho[2,3-b]furan-4,9-dione.

Non-limiting examples of prodrugs of compounds having formula A include, for example, the phosphoric esters and phosphoric diesters described in U.S. pre-grant Publication No. 2012/0252763 as compound numbers 4011 and 4012 and also suitable compounds described in U.S. Patent No. 9,150,530. Non-limiting examples of derivatives of compounds having formula A include, for example, the derivatives disclosed in U.S. Patent No. 8,977,803. The disclosures of U.S. pre-grant Publication No. 2012/0252763 and U.S. Patent Nos. 9,150,530 and 8,977,803 are incorporated by reference herein in their entireties for any purpose.

Suitable methods of preparing 2-acetylnaphtho[2,3-b]furan-4,9-dione, including its crystalline forms and additional cancer stemness inhibitors, are described in the co-owned PCT applications published as WO 2009/036099, WO 2009/036101, WO 2011/116398, WO 2011/116399, and WO 2014/169078; the contents of each of these applications are incorporated by reference herein by reference in their entireties for any purpose.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, atovaquone, a hydroxy naphthoquinone having the structure of



or prodrugs, derivatives, pharmaceutically acceptable salts or solvates thereof.

In certain embodiments, atovaquone can downregulate cell-surface expression of glycoprotein 130 (gp130), which is required for the activation of the cancer stemness gene, STAT3 (see, for example, WO 2015050844 A1, the content of which is hereby incorporated by reference in its entirety for any purpose).

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with an RNA interfering agent that targets a cancer stemness gene, e.g. STAT3 aiRNA. U.S. Patent No. 9,328,345 provides exemplary asymmetric interfering RNA duplexes (aiRNA) and uses thereof to silence STAT3 expression and treat cancer. The disclosure of U.S. Patent No. 9,328,345 is hereby incorporated by reference herein in its entirety for any purpose In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of a STAT3 aiRNA.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with 2-acetylnaphtho[2,3-b]furan-4,9-dione that targets the cancer stemness gene, e.g. STAT3. In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, in combination with 2-acetylnaphtho[2,3-b]furan-4,9-dione wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of 2-acetylnaphtho[2,3-b]furan-4,9-dione.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with atovaquone that downregulates cell-surface expression of glycoprotein 130 (gp130), which is required for the activation of the cancer stemness gene, STAT3. In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of atovaquone.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, an inhibitor of a cancer stem cell pathway kinase (CSCPK) such as for example, STK33, MELK, AXL, p70S6K, and PDGFRα.

In certain embodiments, an inhibitor of a cancer stem cell pathway kinase (CSCPK) can be, for example, an inhibitor, and derivatives thereof, that inhibit STK33, disclosed in U.S. Patent No. 9,187,454, which is hereby enclosed herein by its entirety for any purpose

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, an aiRNA that targets one or more cancer stem cell pathway kinases (CSCPK). In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of a CSCPK.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, a cancer stem cell pathway kinase aiRNA that targets STK33. In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of a cancer stem cell pathway kinase aiRNA that targets STK33.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, a cancer stem cell pathway kinase aiRNA that targets MELK. In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of a cancer stem cell pathway kinase aiRNA that targets MELK.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, a cancer stem cell pathway kinase aiRNA that targets AXL. In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of a cancer stem cell pathway kinase aiRNA that targets AXL.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, a cancer stem cell pathway kinase aiRNA that targets p70S6K. In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS

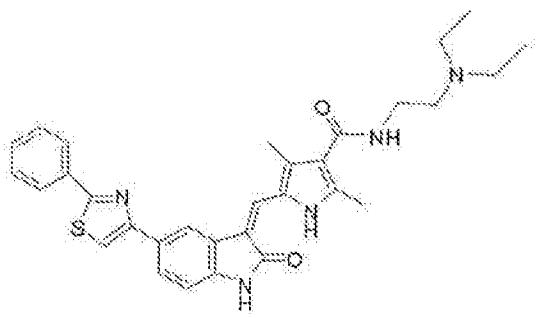
aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of a cancer stem cell pathway kinase aiRNA that targets p70S6K.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, a cancer stem cell pathway kinase aiRNA that targets PDGFR. In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of a cancer stem cell pathway kinase aiRNA that targets PDGFR.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, a cancer stem cell pathway kinase aiRNA that targets NANOG. In certain embodiments, the present disclosure provides a method of treating cancer comprising administering a modulator of KRAS signaling, e.g., a KRAS aiRNA, wherein the modulator of KRAS signaling changes (e.g., enhances) the efficacy of a cancer stem cell pathway kinase aiRNA that targets NANOG.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, a cancer stem cell pathway kinase inhibitor including, but not limited to, compounds, or derivatives thereof, disclosed in U.S. Patent No. 8,299,106 and PCT Patent Application Publication No. WO2014160401. The disclosures of U.S. Patent No. 8,299,106 and PCT Patent Application Publication No. WO2014160401 are incorporated by reference herein by reference in their entireties for any purpose.

In certain embodiments, a modulator of KRAS signaling, e.g., a KRAS aiRNA, as defined herein, can be combined with, for example, a cancer stem cell pathway kinase inhibitor chosen from compounds having formula B, shown below,



(B)

or prodrugs, derivatives, pharmaceutically acceptable salts or solvates thereof.

The present disclosure reports on the surprising discovery that a treatment combination of at least one modulator of KRAS signaling and a therapeutic agent have a greater effect in inhibiting cancer cells than the added effects of both the at least one modulator of KRAS signaling and the at least one therapeutic agent alone.

The present disclosure reports on the surprising discovery that a treatment combination of at least one modulator of KRAS signaling and an immune checkpoint inhibitor have a greater effect in inhibiting cancer cells than the added effects of both the at least one modulator of KRAS signaling and the at least one immune checkpoint inhibitor alone.

The present disclosure reports on the surprising discovery that a treatment combination of at least one modulator of KRAS signaling and an immunotherapeutic agent have a greater effect in inhibiting cancer cells than the added effects of both the at least one modulator of KRAS signaling and the at least one immunotherapeutic agent alone.

The present disclosure reports on the surprising discovery that a treatment combination of at least one modulator of KRAS signaling and at least one cancer stemness inhibitor have a greater effect in inhibiting cancer cells than the added effects of both the at least one modulator of KRAS signaling and the at least one cancer stemness inhibitor alone.

The present disclosure reports on the surprising discovery that a treatment combination of at least one modulator of KRAS signaling and at least one cancer stem cell pathway kinase inhibitor have a greater effect in inhibiting cancer cells than the added effects of both the at least one modulator of KRAS signaling and the at least one cancer stem cell pathway kinase inhibitor alone.

The compositions disclosed herein may be in the form of a pharmaceutical composition. The present disclosure further provides for a pharmaceutical composition comprising an RNA interfering agent, e.g. an aiRNA. The pharmaceutical comprises (as an active agent) at least one RNA interfering agent, e.g., an asymmetrical duplex RNA molecule. In certain embodiments, the pharmaceutical compositions may comprise a modulator of KRAS signaling, e.g. a KRAS aiRNA, and at least one immunotherapeutic agent. In certain embodiments, the pharmaceutical compositions may comprise a modulator of KRAS signaling, e.g. a KRAS aiRNA, and at least one immune checkpoint inhibitor. In certain embodiments, the pharmaceutical compositions may comprise a modulator of KRAS signaling, e.g. a KRAS aiRNA, and at least one cancer stemness inhibitor. In certain embodiments, the pharmaceutical

compositions may comprise a modulator of KRAS signaling, e.g. a KRAS aiRNA, and at least one cancer stem cell pathway kinase (CSCPK) inhibitor.

In certain embodiments, the pharmaceutical compositions may comprise a modulator of KRAS signaling, e.g. a KRAS aiRNA, and one or more compounds and at least one pharmaceutically acceptable carrier, where the one or more compounds are capable of being converted into the at least one therapeutic agent, for example, a cancer stemness inhibitor in a subject, a cancer stem cell pathway kinase (CSCPK) in a subject or an immunotherapeutic agent in a subject (i.e., a prodrug).

In some embodiments, the pharmaceutical composition comprises one or more carriers selected from the group consisting of a pharmaceutical carrier, a positive-charge carrier, a liposome, a protein carrier, a polymer, a nanoparticle, a nanoemulsion, a lipid, and a lipoid. In some embodiments, the composition may comprise more than RNA interfering agent species for therapeutic applications. In certain embodiments, the RNA interfering agent species may target different genes required for cancer initiation and progression chosen from, for example, a modulator of KRAS signaling as disclosed herein, in combination with RNA interfering agent(s) that target one or more cancer stemness genes and/or cancer stem cell kinase as disclosed herein.

The pharmaceutical compositions and formulations of the present disclosure can be the same or similar to the pharmaceutical compositions and formulations developed for siRNA, miRNA, and antisense RNA, except for the RNA ingredient. For example, the siRNA, miRNA, and antisense RNA in the pharmaceutical compositions and formulations can be replaced by the aiRNA molecules of the present disclosure. The pharmaceutical compositions and formulations can also be further modified to accommodate the RNA interfering agent, e.g. aiRNA, of the present disclosure.

A "pharmaceutically acceptable salt" or "salt" of an RNA interfering agent, e.g. aiRNA, is a product of the disclosed RNA interfering agent that contains an ionic bond, and is typically produced by reacting the disclosed RNA interfering agent with either an acid or a base, suitable for administering to a subject. Pharmaceutically acceptable salt can include, but is not limited to, acid addition salts including hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates, and tartrates; alkali metal cations such as Na, K, Li, alkali earth metal salts such as Mg or Ca, or organic amine salts.

A "pharmaceutical composition" is a formulation containing the disclosed RNA interfering agent, e.g. aiRNA, in a form suitable for administration to a subject. In one embodiment, the pharmaceutical composition is in bulk or in unit dosage form. The unit dosage form is any of a variety of forms, including, for example, a capsule, an IV bag, a tablet, a single pump on an aerosol inhaler, or a vial. The quantity of active ingredient *(e.g.,* a formulation of the disclosed duplex RNA molecule or salts thereof) in a unit dose of composition is an effective amount and is varied according to the particular treatment involved. One skilled in the art will appreciate that it is sometimes necessary to make routine variations to the dosage depending on the age and condition of the patient. The dosage will also depend on the route of administration. A variety of routes are contemplated, including oral, pulmonary, rectal, parenteral, transdermal, subcutaneous, intravenous, intramuscular, intraperitoneal, intranasal, and the like. Dosage forms for the topical or transdermal administration of a RNA interfering agent, e.g. aiRNA, of this disclosure include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. In one embodiment, the active RNA interfering agent, e.g. aiRNA, is mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that are required.

The present disclosure provides a method of treatment comprising administering an effective amount of the pharmaceutical composition to a subject in need thereof. In some embodiments, the pharmaceutical composition is administered via a route selected from the group consisting of iv, sc, topical, po, and ip. In another embodiment, the effective amount is 1 ng to 1 g per day, 100 ng to 1 g per day, or 1 ug to 1 mg per day.

The present disclosure also provides pharmaceutical formulations comprising a RNA interfering agent, e.g. aiRNA, of the present disclosure in combination with at least one pharmaceutically acceptable excipient or carrier. As used herein, "pharmaceutically acceptable excipient" or "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in "Remington: The Science and Practice of Pharmacy, Twentieth Edition," Lippincott Williams & Wilkins, Philadelphia, PA., which is incorporated by reference herein by reference. Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional

media or agent is incompatible with the active RNA interfering agent, e.g. aiRNA, use thereof in the compositions is contemplated. Supplementary active RNA interfering agents, e.g. aiRNAs, can also be incorporated into the compositions.

An RNA interfering agent, e.g. aiRNA, of the present disclosure is administered in a suitable dosage form prepared by combining a therapeutically effective amount *(e.g.,* an efficacious level sufficient to achieve the desired therapeutic effect through inhibition of tumor growth, killing of tumor cells, treatment or prevention of cell proliferative disorders, etc.) of a RNA interfering agent, e.g. aiRNA, of the present disclosure (as an active ingredient) with standard pharmaceutical carriers or diluents according to conventional procedures (i.e., by producing a pharmaceutical composition of the disclosure). These procedures may involve mixing, granulating, compressing, or dissolving the ingredients as appropriate to attain the desired preparation. In another embodiment, a therapeutically effective amount of a RNA interfering agent, e.g. aiRNA, of the present disclosure is administered in a suitable dosage form without standard pharmaceutical carriers or diluents.

Pharmaceutically acceptable carriers include solid carriers such as lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary liquid carriers include syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time-delay material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or with a wax, ethylcellulose, hydroxypropylmethylcellulose, methylmethacrylate or the like. Other fillers, excipients, flavorants, and other additives such as are known in the art may also be included in a pharmaceutical composition according to this disclosure.

The term "solvate" represents an aggregate that comprises one or more molecules of a compound of the present disclosure with one or more molecules of a solvent or solvents. Solvates of the compounds of the present disclosure include, for example, hydrates.

The pharmaceutical compositions containing active RNA interfering agent, e.g. aiRNA, of the present disclosure may be manufactured in a manner that is generally known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. Pharmaceutical compositions may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and/or auxiliaries which facilitate processing of the

active duplex RNA molecules into preparations that can be used pharmaceutically. Of course, the appropriate formulation is dependent upon the route of administration chosen.

An RNA interfering agent, e.g. aiRNA, or pharmaceutical composition of the disclosure can be administered to a subject in many of the well-known methods currently used for chemotherapeutic treatment. For example, for treatment of cancers, a RNA interfering agent, e.g. aiRNA, of the present disclosure may be injected directly into tumors, injected into the blood stream or body cavities, taken orally, or applied through the skin with patches.

In certain embodiments, aiRNA nanoparticles may be formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "dosage unit form" as used herein refers to a physically discrete unit of nanoparticle appropriate for the patient to be treated. It will be understood, however, that the total daily usage of the compositions of the present disclosure will be decided by the attending physician within the scope of sound medical judgment. For any nanoparticle, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. Therapeutic efficacy and toxicity of nanoparticles can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose is therapeutically effective in 50% of the population) and LD50 (the dose is lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices may be useful in some embodiments. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for human use.

Compositions suitable for parenteral administration may comprise at least one more pharmaceutically acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions, emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

When *in vivo* administration of the polypeptides or antibodies described herein are used, normal dosage amounts may vary from about 10 ng/kg up to about 100 mg/kg of mammal body weight or more per day, preferably about 1 mg/kg/day to 10 mg/kg/day, depending upon the

route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. No. 4,657,760; 5,206,344; or 5,225,212, the contents of which are hereby incorporated by reference herein in its entireties for any purpose.

In various embodiments, a composition described herein includes at least one compound and pharmaceutically acceptable salts and solvates thereof and one or more surfactants. In certain embodiments, the surfactant is sodium lauryl sulfate (SLS), sodium dodecyl sulfate (SDS), or one or more polyoxylglycerides. For example, the polyoxylglyceride can be lauroyl polyoxylglycerides (sometimes referred to as Gelucire™) or linoleoyl polyoxylglycerides (sometimes referred to as Labrafil™). Examples of such compositions are disclosed in PCT Patent Application No. PCT/US2014/033566, the content of which is incorporated by reference herein in its entirety for any purpose.

The present disclosure provides further embodiments of suitable pharmaceutical formulations having selected particle size distribution and methods for identifying an optimum particle size distribution, suitable drug regimen, dosage and interval, suitable methods of preparing 2-acetylnaphtho[2,3-*b*]furan-4,9-dione including their crystalline forms, and further specific suitable cancer stemness inhibitors and kinase inhibitors as described in the co-owned PCT applications published as WO 2009/036099, WO 2009/036101, WO 2011/116398, WO 2011/116399, WO 2014/169078, and WO 2009/033033, the contents of which are hereby incorporated by reference herein in their entireties for any purpose.

In certain embodiments, a kit is disclosed that comprises (1) at least one compound chosen from a modulator of KRAS signaling and (2) an immune checkpoint inhibitor, an immunotherapeutic, a chemotherapeutic agent, a cancer stemness inhibitor, a cancer stem cell pathway kinase inhibitor or prodrugs thereof, derivatives thereof, pharmaceutically acceptable salts of any of the foregoing, together with instructions for administration and/or use.

EXAMPLES

Examples are provided below to further illustrate different features of the present disclosure. The examples also illustrate useful methodology for practicing the disclosure. These examples do not limit the claimed disclosure.

EXAMPLE 1: KRAS SILENCING IS SUFFICIENT TO DEPLETE PD-L1 EXPRESSION IN KRAS MUTANT CELLS

Asymmetric interfering RNA (aiRNA) silences target genes potently and specifically with minimal off-target effects. aiRNAs targeting KRAS were therefore used to determine the effects of KRAS depletion on PD-L1 expression in KRAS mutant MDA-MB-231 cells expressing endogenous PD-L1.

aiRNAs were first synthesized in DMT-on mode. Following completion of the synthesis, the solid support was suspended in 600 µl EtOH/NH_4OH solution (prepared by mixing 1 volume of 200 proof ethanol with 3 volumes of 28% NH_4OH) and heated at 55 °C for 2 hours. After primary de-protection, EtOH/NH_4OH was evaporated and the RNA oligo was dried to a pellet. 100µl of RNA de-protection solution (NMP/TEA.3HF (3:2)) was added and the solution was heated at 65 °C for 1.5 hours. The reaction was then quenched with 400 µl of 1.5 M ammonium bicarbonate. Purification was performed with Clarity® QSP Cartridges (Phenomenex, USA). The annealing of the resulting duplexes was confirmed on 15% PAGE gel. All sequences are shown in FIG. 1A. The location of each of the sequences targeted by aiKRAS is shown in FIG. 1B.

The human breast adenocarcinoma cell line, MDA-MB-231, that is heterozygous for the KRAS G13D mutation, was obtained from ATCC (ATCC® CRM-HTB-26™) and maintained in DMEM supplemented with 10% (vol/vol) FBS (Gemini Bio Products, USA) and 1% penicillin/streptomycin (Life Technologies, USA).

MDA-MB-231 cells were then transfected with multiple aiRNAs targeting KRAS (aiKRAS#1-#4). Cells were seeded to 60mm plates (2 x 10^5 cells/4 mL/well). aiRNA was transfected by Lipofectamine® RNAiMAX (Thermo Fisher, USA) according to the manufacturer's protocol. aiRNA and RNAiMAX were incubated for 20 min in serum free OPTI-MEM (Thermo Fisher, USA), then added to the cells along with culture medium. 48 hours after transfection, cells were harvested using Accutase® cell detachment solution (Sigma Aldrich, USA). Scrambled aiRNA was used as a control. The amount of PD-L1, KRAS and actin protein was then determined by Western blot analysis.

The cells were washed twice with ice cold PBS and lysed in lysis buffer [50 mM Hepes (pH 7.5), 1% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, and 1× protease inhibitor mixture (EMD Millipore, USA)]. Soluble protein (20 µg) was separated by SDS/PAGE and transferred to PVDF membranes and probed with commercially available antibodies specific for actin (Sigma Aldrich, USA), KRAS and PD-L1 (Santa Cruz Biotechnology, Inc., USA).

As shown in FIG. 2A, transfection of the KRAS aiRNAs resulted in a marked reduction in the amount of PD-L1 even at concentration of 100pM (Fig. 1A).

The amount of KRAS and PD-L1 mRNA in the aiKRAS RNA transfected cells was then determined by quantitative RT-PCR. Cells transfected with aiKRAS#1-#4 or scrambled control aiRNA, as described above, were seeded in six-well plates with culture media. At the appropriate time points, cells were harvested and RNA extracted using the SimplyRNA kit (Promega, USA) according the manufacturer's protocol. Reverse transcription was performed on 1μg of RNA from each sample using the GoScript reverse transcription kit (Promega, USA). Real-time PCR was carried out using the RT2 qPCR Primer Assays (SABiosciences, USA) except for KRAS. Primers of KRAS were synthesized by Integrated DNA Technologies, USA. KRAS primer sequences were as follows: 5'-CCTACTAGGACCATAGGTACATCT-3' (SEQ ID NO.: 982) and 5'-AGGGCTTTCTTTGTGTATTTGC-3' (SEQ ID NO.: 983). Genes of interest were normalized to the expression of β-2-microglobulin (B2M).

As shown in FIG. 2B, the level of PD-L1 mRNA was also down-regulated by KRAS silencing with aiKRAS RNAs 1-4. These results suggested that KRAS silencing down-regulated PD-L1 expression in a transcription factor dependent manner. Time course experiments showed that aiRNA mediated KRAS silencing was induced very quickly and reached maximum activity 8 hours post-transfection whereas PD-L1 was down-regulated only after 32 hours post-transfection (Fig. 2C) suggesting the presence of intermediate steps between KRAS silencing and the subsequent reduction in PD-L1 mRNA levels.

To investigate the dependency of PD-L1 downregulation on KRAS mutation status, three KRAS mutant cancer cell lines (H358, H460, and H2009) and two KRAS wild-type cancer cell lines (RKO and TCCSUP) were transfected with aiKRAS#1 or aiControl (FIG. 2D). As observed earlier in MDA-MB-231 cells, PD-L1 expression was down-regulated with KRAS depletion in KRAS mutant cancer cells at both the protein and mRNA level (Fig. 2D and Fig. 2E). In contrast, PD-L1 expression protein expression in KRAS wild-type cancer cells was not altered, even though KRAS was potently silenced by aiRNA (Fig. 2D and 2E). Thus oncogenic KRAS mutant but not wild-type KRAS is essential to maintain PD-L1 expression in KRAS mutant cancer cells.

EXAMPLE 2: KRAS MAINTAINS PD-L1 EXPRESSION THROUGH ENDOGENOUSLY ACTIVATED MEK/ERK PATHWAY

Mutant KRAS activates various down-stream signaling pathways including the RAF/MEK/ERK pathway, the PI3K/AKT pathway, and the RalGTP pathway. To determine the effect of KRAS depletion on downstream signaling events, aiKRAS or aiControl transfected KRAS mutant MDA-MB-231 and KRAS wild-type RKO cells were washed twice with ice cold PBS and lysed in lysis buffer [50 mM Hepes (pH 7.5), 1% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, and 1× protease inhibitor mixture (EMD Millipore, USA)]. Soluble protein was then probed using a Human Phospho-Kinase Antibody Array according to the manufacturer's instructions (R&D Systems; Catalog # ARY003B). Among the anti-phosphokinase antibodies spotted on the array, phosphorylated ERK1/2 was most prominently down-regulated with KRAS depletion in KRAS mutant MDA-MB-231 cells but not in KRAS wild-type RKO cells (Fig. 3A).

To confirm the effect of KRAS silencing on the RAF/MEK/ERK pathway, the phosphorylation state of MEK/ERK was determined by Western blot. Soluble protein (20 µg) from aiKRAS or aiControl transfected KRAS mutant MDA-MB-231 and KRAS wild-type RKO cell lysates was separated by SDS/PAGE and transferred to PVDF membranes and probed with actin (Sigma Aldrich, USA) KRAS (Santa Cruz Biotechnology, Inc., USA), Phospho-MEK1/2, MEK1/2, Phospho-p44/42 MAPK (Erk1/2), p44/42 MAPK (Erk1/2) antibodies (Cell Signaling Technology, Inc., USA). As shown in FIG. 4A, KRAS silencing downregulated the phosphorylation of both MEK and ERK only in KRAS mutant MDA-MB-231 cells but not in RKO cells.

To explore further the role of the RAF/MEK/ERK pathway on PD-L1 expression, KRAS mutant MDA-MB-231 cells were treated with the MEK-specific inhibitor, U0126. Similar to aiKRAS, U0126 also down-regulated PD-L1 expression in MDA-MB-231 cells and inhibited ERK phosphorylation (Fig. 4B). These results indicate the ERK pathway is involved in KRAS dependent PD-L1 expression in KRAS mutant cancer cells.

EXAMPLE 3: KRAS MAINTAINS PD-L1 EXPRESSION THROUGH ENDOGENOUSLY ACTIVATED AP1

ELISA based detection of AP-1 transcription factor activation

The down-regulation of PD-L1 mRNA levels by KRAS silencing suggested that PD-L1 expression levels were maintained by a transcriptional factor regulated by mutant KRAS. Among the transcriptional factors regulated by RAS and ERK, one of the most well studied

transcriptional factors is Activator protein 1 (AP-1). The AP-1 transcriptional factor is formed by either the homo-dimerization of a Jun family protein (cJun, JunB, or JunD) or by the more stable hetero-dimerization of a Jun family protein with a Fos family protein (cFOS, FOSB, FRA-1, or FRA-2). In RAS activated cells, the accumulation of stabilized FRA-1 was reported to be dependent on phosphorylation by ERK1/2.

To investigate the effect of KRAS depletion on FRA-1 expression, ai-KRAS#1 was transfected into KRAS mutant cancer cell lines (MDA-MB-231, H358, and H460) and a KRAS wild-type cancer cell line (RKO). Immunoblotting analysis with a Phospho-FRA-1 and FRA-1 antibody (Cell Signaling Technology, Inc., USA) showed that KRAS depletion down-regulated the phosphorylated and total forms of FRA-1 in MDA-MB-231, H358, and H460 cells harboring KRAS mutations but not in KRAS wild-type RKO cells (Fig.5A). This result confirmed that FRA-1 is a KRAS dependent transcriptional factor as reported previously by Casalino *et al.* (2003).

The effect of KRAS silencing on DNA binding activity of FRA-1 was then quantified by ELISA using the Trans-AM AP-1 transcription factor assay (Active Motif, USA). Nuclear extract was prepared with NE-PER® Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher, USA). 5μg of nuclear extracts were incubated with coated immobilized oligonucleotides containing a 12-O-tetradecanoylphorbol-13-acetate (TPA) response element (TRE) with the 5'-TGA(C/G) TCA-3' (SEQ ID NO.: 984) sequence. To ensure the specificity of the assay, 20pM of consensus oligonucleotides were incubated in parallel as competition experiments.

As shown in FIG. 5B, KRAS silencing reduced the endogenous FRA-1 binding activity in KRAS mutant MDA-MB-231 cells but not in KRAS wild-type RKO cells. This result was consistent with the observed down-regulation of FRA-1 expression by KRAS silencing in KRAS mutant cells (Fig. 4A).

Intron 1 of the PD-L1 gene, located approximately 5kb downstream from the transcription start site, contains a conserved candidate enhancer element with putative AP1 binding sites. To determine if FRA-1 could bind to the putative PD-L1 enhancer in a KRAS dependent manner, a chromatin immunoprecipitation (ChIP) quantified PCR assay was performed in the presence of anti-phospho-Fra-1 or cJun antibody using SimpleChIP® Enzymatic Chromatin IP Kit (Cell signaling Technologies, USA) according to the manufacturer's protocol. aiRNA transfected MDA-MB-231 cells were cross-linked by

treatment with 1% formaldehyde in culture medium for 10min at room temperature. After washing with cold PBS, nuclear pellets were prepared by treating the cells with Buffers A and B. The nuclear pellets were then suspended in buffer B and treated with SDS buffer and micrococcal nuclease to digest chromatin for 20 min at 37°C. Cell suspensions were then sonicated to elute nuclear chromosomal content in preparation for immunoprecipitation. 2 µg each of Phospho-FRA-1 and cJun were incubated with the lysate overnight at 4°C. Immunocomplexes were collected with magnetic protein A beads and eluted after extensive washing. Cross-linkage was reversed by heating the solution at 65°C with NaCl and proteinase K. DNA was purified and used as the template for quantitative PCR to amplify the region including the AP-1 binding site. The primer pairs used were 5'-GTCACATTTCAAGCAGGATGACTA-3' (SEQ ID NO.: 985) and 5'-GGAAGGGGAGAGAG TTGGATT-3' (SEQ ID NO.: 986). Quantitative PCR was performed as described in Example 1.

As shown in FIG. 5C (left side), both FRA-1 and cJUN were recruited to candidate PD-L1 enhancer sites in MDA-MB-231 cells. Binding of FRA-1 and cJUN to the PD-L1 enhancer decreased with KRAS depletion. In contrast, as with PD-L1 gene expression, FRA-1 and cJUN binding to the PD-L1 enhancer was not altered by KRAS depletion in KRAS wild-type RKO cells (Fig. 5C, right side).

To determine the effect of FRA-1 silencing on PD-L1 expression, aiFra-1#1, aiFra-1#2 (see FIG. 1A) were transfected into KRAS mutant MDA-MB-231 and KRAS wild-type RKO cells. Immunoblotting and quantitative RT-PCR confirmed that transfection of aiFra-1#1 or #2 resulted in depletion of FRA-1 protein and mRNA (FIG. 6 A and 6B). Moreover, knockdown of FRA-1 also resulted in a marked down-regulation of PD-L1 protein and mRNA expression (Fig. 6A and 6B).

STAT3 or NF-KB are also known to promote the expression of PD-L1 mRNA by binding directly to the PD-L1 promoter region. However, unlike as with FRA-1, KRAS silencing did not alter the expression of either STAT3 or NF-KB (see FIGs. 4C and 4D).

These results indicate that PD-L1 expression was maintained in KRAS mutant cells via the RAS/ERK/Fra-1 signaling pathway (see FIG. 8B).

EXAMPLE 4: KRAS SILENCING IS SUFFICIENT TO RESTORE SENSITIVITY OF KRAS MUTANT CANCER CELLS TO ANTI-PD-L1 OR TO CTL KILLING

The functional significance of these results on cancer immune evasion was assessed using luciferase expressing KRAS mutant MDA-MB-231 cells transfected with control aiRNA or aiKRAS#1.

Luc-MDA-MB-231, a cell line stably expressing luciferase, was purchased from Cell Biolabs, Inc., USA. Luc-MDA-MB-231 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% inactivated fetal calf serum (FCS) and transfected with either control aiRNA or aiKRAS#1 as described in Example 1.

Cell surface expression of PD-L1 in the control aiRNA or aiKRAS#1 transfected Luc-MDA-MB-231 cells was determined by staining the cells with PE conjugated anti-CD274 (BD Biosciences, USA) in Stain Buffer (BD Biosciences, USA) on ice for 20 min and washed once with Stain Buffer. CD274 positive population was then detected using flow cytometry (Attune Acoustic Focusing Cytometer, Life Technologies, USA) and analyzed with Flowjo software (Flowjo, LLC, USA). Flow cytometry analysis confirmed that KRAS silencing down-regulated cell surface PD-L1 expression (Fig. 7B).

To determine if KRAS silencing altered T cell cytotoxicity, aiRNA-transfected MDA-MB-231 cells were either pulsed or not pulsed with CMV antigen peptide, and then co-cultured with CMV specific cytotoxic T lymphocytes (CTLs) expanded from HLA typed human PBMC from healthy donors.

HLA typed PBMCs from healthy donor were purchased from Cellular Technology, Ltd., USA. PBMCs were diluted at 5 x 10^6 cells/mL in culture medium (RPMI-1640 supplemented with 10% inactivated human serum and 50 μM 2-mercaptoethanol) and seeded into 24-well plates (5 x 10^6 cells/mL/well). HLA-A*02:01 CMV pp65 peptide (NLVPMVATV) (IBA Lifesciences, Germany) was added to a final concentration of 5 μM on day 0 as well as 25 IU/mL IL-2 (R&D Systems, USA) and 5 ng/mL IL-15 (R&D Systems, USA). The cultures were supplemented with fresh medium containing 10 μM HLA-A*02:01 CMV pp65 peptide, 50 IU/mL IL-2 and 10 ng/mL IL-15 every 3-4 days. $CD8^+$ T cells were isolated from PBMC using a $CD8^+$ T cell Isolation Kit (Miltenyi Biotec, USA) as described by the manufacturer's protocol.

The aiRNA transfected Luc-MDA-MB-231 cells were incubated with or without HLA-A*02:01 CMV pp65 peptide (NLVPMVATV) for 2 hours at 37°C in 5% CO_2 and then washed twice with PBS. CMV peptide loaded or unloaded Luc-MDA-MB-231 cells were plated into 96-well plates (2000 cells/well). CMV specific $CD8^+$ T cells were subsequently added to a 96-well plate with an Effector: Target (E/T) ratio of 50:1 and incubated for 24 hours. Anti-Human PD-L1 Ab at a final concentration of 10μg/mL was used as a control (Clone: MIH1, Functional grade purified, Affymetrix eBioscience, USA). Live Luc-MDA-MB-231 cells were measured by intracellular luciferase activity with XenoLight D-Luciferin K^+ salt (PerkinElmer, USA) by GloMax® Discovery (Promega, USA). The percent Lysis was calculated with the formula: (Luminescence of CMV peptide pulsed Luc-MDA-MB-231/ Luminescence of CMV peptide un-pulsed Luc-MDA-MB-231)*100.

As shown in FIG. 7A, the cytotoxic activity of antigen specific CTLs were significantly enhanced with aiKRAS transfection ($p<0.01$) compared with scramble aiRNA transfected cells. To validate PD-L1 dependency of the observed cytotoxic activity, aiRNA targeting PD-L1 was compared to the activity of a commercially available functional grade anti-PD-L1 antibody. Both treatments enhanced CTL killing activity against MDA-MB-231 cells (Fig. 7A) to a level that was comparable to the cytotoxic activity seen with KRAS depletion.

The results support the conclusion that KRAS activates immune evasion of cancer cells by maintenance of constitutive PD-L1 expression in the cancer cells.

EXAMPLE 5: PREPARATION OF KRAS aiRNA NANOPARTICLES (NPs)

aiRNAs were synthesized in DMT-on mode as described in Example 1. For certain applications, selected nucleotides in the aiRNAs were modified with a 2'-OH methyl group.

Nanoparticles (NPs) loaded with aiRNA were prepared using the double-emulsion process described in WO2014123935, the content of which is hereby incorporated by reference herein in its entirety.

Specifically, in each of vials A and B, 5 mL of cyclohexane/Igepal CO-520 solution (71/29 v/v) were prepared from reagents respectively available from EMD and Sigma.

aiRNA was dispersed in 1x RNAse-free buffer to make the desired concentration (e.g., about 5 μg/μl). A volume of aiRNA (e.g., about 50 μg) was mixed with 100 μL of 500 mM $MgCl_2$. Then, the $MgCl_2$-aiRNA solution was added drop-wise to the oil/surfactant solution in vial A to form a well-dispersed emulsion without reverse micro-emulsion.

In vial B, 100 µL of 25 mM Na_2HPO_4 (pH=9) was added drop-wise to the oil/surfactant solution. The contents of vials A and B were then mixed and stirred for 30 minutes at room temperature. Afterwards, the contents were transferred into 10 centrifuge tubes (1.5 mL) and centrifuged for 30 minutes at 13,000g. The supernatant was discarded and the pellet was washed with absolute ethanol (1 mL) twice. After the alcohol was removed, the resulting pellet was air-dried for 3-4 hours.

A polymer-based shell was coated onto the MgP nanoparticle cores already loaded with aiRNA. Specifically, biodegradable polymers, PEG(5k)-Poly-L-Lysine (10U), Poly-L-Arginine (50U) were coated onto the cores at a polymer ratio of 2.5:1 (PLL: PLR) & a complex ratio of 2.5:1 (polymer: aiRNA).

Measurement for size/PdI determination of liposomes was performed. The average size of the nanoparticles was about 70 nm and the surface charge was about +25 mV. The nanoparticles exhibited good plasma stability, cellular uptake and endosomal escape.

EXAMPLE 6: INHIBITION OF KRAS SIGNALING ENHANCES THE THERAPEUTIC EFFICACY OF RADIATION THERAPY

The human breast adenocarcinoma cell line, MDA-MB-231 (ATCC® CRM-HTB-26™) is cultured in DMEM supplemented with 10% (vol/vol) FBS (Gemini Bio Products, USA) and 1% penicillin/streptomycin (Life Technologies, USA). Approximately, 6 X 10^6 cells /mouse are then inoculated subcutaneously into female athymic nude mice.

Xenografted mice are randomized into the following treatment groups:

Group 1: Control aiRNA NP

Group 2: Radiotherapy + saline

Group 3: aiKRAS NP + Control aiRNA NP

Group 4: aiKRAS NP + Radiotherapy

Irradiations are performed 7-10 days after inoculation (when tumors were at least 100 mm^3) using a Pantak HF-320 320 kV x-ray unit (Gulmay Medical, U.K.). The machine is operated at 300 kV, 9.2 mA, with filtration fitted in the x-ray beam to give a radiation quality of 2.3 mm Cu half-value layer. Mice are positioned at a distance of 350 mm from the x-ray focus, where the dose rate is 0.80 Gy/min.

The intravenous administration of nanoparticle (NP) preparations comprising either KRAS aiRNAs or an aiRNA control commences on day 1 of the fractionated radiation therapy cycle and is repeated every other day for 8 days (at a dose of 2.5 mg/kg/NP preparation). The treatment is well tolerated with minimal adverse side effects. The size of the tumors is measured every day. Experimental groups contain at least 5 mice/group and are representative of at least 2 independent experiments.

Low doses of local fractionated dose radiotherapy are delivered at about 10 Gy in 5 fractions leads to increased tumor cell expression of PD-L1 with elevated expression evident 1, 3 and 5 days after the last dose of radiotherapy when compared to time-matched mice receiving the control aiRNA NP. Radiotherapy increases tumor cell PD-L1 expression which peaks at about 72 hours after the last dose of radiation and remains elevated as compared to non-treated control mice.

The radiotherapy-mediated local tumor control is improved through the administration of aiKRAS#1-#4 nanoparticle formulation. However, a synergistic anti-tumor response with a T/C value < 10% is observed in mice that receive radiotherapy in combination with the aiKRAS#1-#4 NP as compared to mice that received radiotherapy in combination with the scrambled control aiRNA formulation.

EXAMPLE 7: INHIBITION OF KRAS SIGNALING ENHANCES THE THERAPEUTIC EFFICACY OF A CHEMOTHERAPEUTIC AGENT

Approximately, 6 X 10^6 human breast adenocarcinoma cells (MDA-MB-231 (ATCC® CRM-HTB-26™)) /mouse are inoculated subcutaneously into female athymic nude mice. Treatment starts when the tumor burden (mouse weight) reaches approximately 20 grams (~21 days after injection of cells).

Xenografted mice are randomized into the following treatment groups:

Group 1: Control aiRNA NP + saline
Group 2: Control aiRNA NP + paclitaxel
Group 3: aiKRAS#1-#4 NP + saline
Group 4: aiKRAS#1-#4 NP + paclitaxel

Nanoparticle (NP) preparations comprising either aiKRAS#1-#4 or an aiRNA control are administered by intravenous injection to the xenografted mice of Groups 1, 3 and 4 starting

on day 1. The aiRNA injections are repeated every other day for 12 days (i.e., a dose of 2.5 mg/kg/NP preparation on day 1, 3, 5, 8, and 10).

Paclitaxel (Taxol®) is administered to Groups 2 and 4 by intraperitoneal injection on day 2, 4, 6, 9 and 11 at a dose of 10 mg/kg preparation.

The treatment is well tolerated with minimal adverse side effects. Xenografted animals are periodically weighed to assess the effect of treatment on tumor burden. Experimental groups contain 10 mice/group and are representative of at least 2 independent experiments.

The intraperitoneal injection of paclitaxel alone or aiKRAS NP alone results in a delay in tumor progression. However, the administration of aiKRAS#1-#4 NP together with paclitaxel to the xenografted mice of Group 4 results in a synergistic anti-tumor response with a T/C value < 10% when compared to time-matched, mice receiving either the aiKRAS NP alone with saline or paclitaxel with control aiRNA NP.

EXAMPLE 8: INHIBITION OF KRAS SIGNALING ENHANCES THE THERAPEUTIC EFFICACY OF AN IMMUNE CHECKPOINT RNA INTERFERING AGENT

Approximately, 6 X 10^6 human breast adenocarcinoma cells (MDA-MB-231 (ATCC® CRM-HTB-26™)) /mouse are inoculated subcutaneously into female athymic nude mice. Treatment starts when the tumor burden (mouse weight) reaches approximately 20 grams (~21 days after injection of cells).

Xenografted mice are randomized into the following treatment groups:

Group 1: Control aiRNA NP
Group 2: aiPD-L1 NP + Control aiRNA NP
Group 3: aiKRAS NP + Control aiRNA NP
Group 4: aiKRAS NP + aiPD-L1 NP

Nanoparticle (NP) preparations comprising either PD-L1 aiRNA (e.g. comprising aiRNA sequences SEQ ID NOs.: 972 and 973), KRAS aiRNA, or an aiRNA control are administered to Groups 1-4 by intravenous injection starting on day 1. The aiRNA injections are then repeated every other day for 12 days, i.e., at a dose of 2.5 mg/kg/NP preparation on day 1, 3, 5, 8, and 10). Xenografted animals are periodically weighed to assess the effect of treatment on tumor burden. The treatment is well tolerated with minimal adverse side effects.

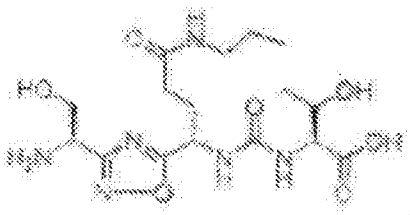
Experimental groups contain 10 mice/group and are representative of at least 2 independent experiments.

Intravenous injection of the aiKRAS NP with control aiRNA NP (Group 3) or aiPD-L1 NP with control aiRNA NP (Group 2) results in a delay of tumor progression. However, the administration of aiKRAS NP together with PD-L1 aiRNA to the xenografted mice of Group 4 results in a synergistic anti-tumor response with a T/C value < 10% when compared to time-matched, mice receiving either the aiKRAS NP with control aiRNA NP (Group 3) or aiPD-L1 NP with control aiRNA NP (Group 2).

EXAMPLE 9: INHIBITION OF KRAS SIGNALING ENHANCES THE THERAPEUTIC EFFICACY OF A SMALL MOLECULE IMMUNE CHECKPOINT INHIBITOR

Approximately, 6 X 10^6 human breast adenocarcinoma cells (MDA-MB-231 (ATCC® CRM-HTB-26™)) /mouse are inoculated subcutaneously into female athymic nude mice. Treatment starts when the tumor burden (mouse weight) reaches approximately 20 grams (~21 days after injection of cells).

The small molecule immune checkpoint inhibitor used in this example is Compound No. 75 of WO2016142833, having the structure of:



Compound Nos.75 rescues the PD-L1 induced inhibition of cell proliferation in a mouse splenocyte assay by 119% (see Table I of WO2016142833).

Xenografted mice are randomized into the following treatment groups:

Group 1: Control aiRNA NP + saline

Group 2: Control aiRNA NP + Compound No. 75

Group 3: aiKRAS NP + saline

Group 4: aiKRAS NP + Compound No. 75

Nanoparticle (NP) preparations comprising either KRAS aiRNA or an aiRNA control are administered to Groups 1-4 by intravenous injection starting on day 1. The aiRNA

injections are then repeated every other day for 12 days, i.e., at a dose of 2.5 mg/kg/NP preparation on day 1, 3, 5, 8, and 10).

Compound No. 75 is administered orally at a daily dose of 10 mg/kg for 12 days.

Xenografted animals are periodically weighed to assess the effect of treatment on tumor burden. The treatment is well tolerated with minimal adverse side effects. Experimental groups contain 10 mice/group and are representative of at least 2 independent experiments.

Administration of either aiKRAS NP with saline or Compound No. 75 with the aiRNA control NP results in a delay in tumor progression. However, the administration of aiKRAS NP together with Compound No. 75 to the xenografted mice of Group 4 results in a synergistic anti-tumor response with a T/C value < 10% when compared to time-matched, mice receiving either the aiKRAS NP with saline or Compound No. 75 with the aiRNA control NP.

EXAMPLE 10: INHIBITION OF KRAS SIGNALING ENHANCES THE THERAPEUTIC EFFICACY OF AN ANTI-PD- L1 ANTIBODY

Mice are inoculated subcutaneously with 0.5 10^6 MC38.Ova cells on Day 0. On Day 1 or on Day 14 mice (when tumors reach an average size of 250 mm^3), the xenografted mice are randomized into the following treatment groups (10 mice/group):

Group 1: Control Ig + Control aiRNA NP

Group 2: Atezolizumab + Control aiRNA NP

Group 3: Control Ig + aiKRAS NP

Group 4: atezolizumab + aiKRAS NP

Nanoparticle (NP) preparations comprising either KRAS aiRNA or an aiRNA control are administered to Groups 1-4 by intravenous injection starting on day 1. The aiRNA injections are then repeated every other day for 14 days, i.e., at a dose of 2.5 mg/kg/NP preparation on day 1, 3, 5, 8, 10 and 12).

Anti-PD-L1 (atezolizumab; Genentech/Hoffmann-La Roche) or control Ig is injected 3×/week for 2 weeks at a dose of 10 mg/kg/NP preparation.

Xenografted animals are periodically weighed to assess the effect of treatment on tumor burden. The treatment is well tolerated with minimal adverse side effects. Experimental groups contain 10 mice/group and are representative of at least 2 independent experiments.

Administration of atezolizumab and control aiRNA NP (Group 2) or control Ig and aiKRAS NP (Group 3) results in a delay in tumor progression. However, the administration of aiKRAS NP together with atezolizumab to the xenografted mice of Group 4 results in a synergistic anti-tumor response with a T/C value < 10% when compared to time-matched, mice receiving either the atezolizumab and control aiRNA NP (Group 2) or control Ig and aiKRAS NP (Group 3).

EXAMPLE 11: INHIBITION OF KRAS SIGNALING ENHANCES THE THERAPEUTIC EFFICACY OF A CANCER STEMMESS INHIBITOR

FaDu human head and neck cancer cells were inoculated subcutaneously into female athymic nude mice (6×106 cells/mouse) and allowed to form palpable tumors. When the tumors reached approximately 100 mm^3, the xenografted mice are randomized into the following treatment groups (10 mice/group):

Group 1: vehicle control + Control aiRNA NP
Group 2: BBI-608+ Control aiRNA NP
Group 3: vehicle control + aiKRAS NP
Group 4: BBI608 + aiKRAS NP

Nanoparticle (NP) preparations comprising either KRAS aiRNA or an aiRNA control are administered to Groups 1-4 by intravenous injection starting on day 1. The aiRNA injections are then repeated every other day for 14 days, i.e., at a dose of 2.5 mg/kg/NP preparation on day 1, 3, 5, 8, 10 and 12).

Animals are treated orally (po) with 2-acetyl-4H,9H-naphtho[2,3-b]furan-4,9-di-one (BBI608) at 100 mg/kg or vehicle control daily. BBI-608 was formulated at 10 mg/ml in 20% gelucire. Suitable methods of preparing 2-acetylnaphtho[2,3-b]furan-4,9-dione, including its crystalline forms, are described in the co-owned PCT applications published as WO 2009/036099, WO 2009/036101, WO 2011/116398, WO 2011/116399, and WO 2014/169078. Xenografted animals are periodically weighed to assess the effect of treatment on tumor burden. The treatment is well tolerated with minimal adverse side effects. Experimental groups contain 10 mice/group and are representative of at least 2 independent experiments.

Administration of BBI608 and control aiRNA NP (Group 2) or vehicle control and aiKRAS NP (Group 3) results in a delay in tumor progression. However, the administration of

aiKRAS NP together with BBI-608 to the xenografted mice of Group 4 results in a synergistic anti-tumor response with a T/C value < 10% when compared to time-matched, mice receiving either the BBI608 and control aiRNA NP (Group 2) or vehicle control and aiKRAS NP (Group 3).

EXAMPLE 12: KRAS SILENCING ENHANCES THE THERAPEUTIC EFFICACY OF CANCER STEM CELL PATHWAY INHIBITOR

SW480 colon cancer cells are inoculated subcutaneously into male athymic nude mice (8×10^6 cells/mouse) and allowed to form palpable tumors. Once the tumors reached approximately 200 mm^3, the xenografted mice are randomized into the following treatment groups (10 mice/group):

Group 1: vehicle control + Control aiRNA NP

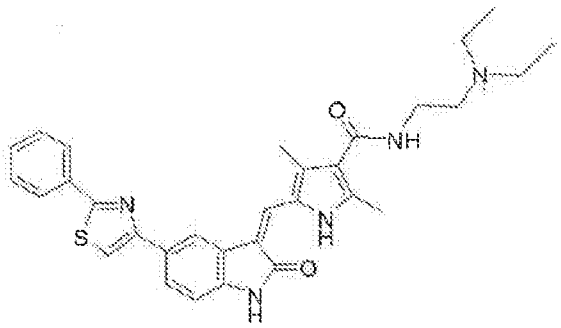
Group 2: BBI503+ Control aiRNA NP

Group 3: vehicle control + aiKRAS NP

Group 4: BBI503 + aiKRAS NP

Nanoparticle (NP) preparations comprising either KRAS aiRNA or an aiRNA control are administered to Groups 1-4 by intravenous injection starting on day 1. The aiRNA injections are then repeated every other day for 14 days, i.e., at a dose of 2.5 mg/kg/NP preparation on day 1, 3, 5, 8, 10 and 12).

Animals are treated orally (po) at 5 mg/kg or vehicle control daily with the cancer stem cell kinase (CSCK) inhibitor (BBI503) having the structure of:



Xenografted animals are periodically weighed to assess the effect of treatment on tumor burden. The treatment is well tolerated with minimal adverse side effects. Experimental groups contain 10 mice/group and are representative of at least 2 independent experiments.

Administration of BBI503 and control aiRNA NP (Group 2) or vehicle control and aiKRAS NP (Group 3) results in a delay in tumor progression. However, the administration of aiKRAS NP together with BBI-503 to the xenografted mice of Group 4 results in a synergistic anti-tumor response with a T/C value < 10% when compared to time-matched, mice receiving either the BBI503 and control aiRNA NP (Group 2) or vehicle control and aiKRAS NP (Group 3).

CLAIMS

1. A composition comprising:

an effective amount of a modulator of oncogenic KRAS signaling, and

an effective amount of an immune checkpoint inhibitor,

wherein the combination of the modulator of oncogenic KRAS signaling and the inhibitor of the immune checkpoint pathway is effective at sensitizing tumor cells in a subject to the immune checkpoint inhibitor.

2. The composition of claim 1, wherein the tumor cells are resistant to the immune checkpoint inhibitor.

3. The composition of claim 1, wherein the tumor cells express an oncogenic KRAS.

4. The composition of claim 1, wherein the immune checkpoint inhibitor is effective at blocking the interaction of programmed cell death protein 1 (PD-1) receptor with programmed cell death 1 ligand 1 (PD-L1).

5. The composition of claim 1, wherein the immune checkpoint inhibitor is chosen from ipilimumab, tremelimumab, atezolizumab, nivolumab, pembrolizumab, JS001, REGN2810, SHR-1210, MEDI0680, PDR001, BGB-A317, TSR-042, PF-06801591, Ningbo Cancer Hosp. anti-PD-1 CAR, Medimmune anti-PD-1, Isis anti-PD-1, UCB anti-PD-1 or 948.g1, Dana-Farber anti-PD-1, STI-1110, Suzhou Stainwei Biotech anti-PD-1, Haixi pembrolizumab biosimilar, Livzon anti-PD-1, MabQuest anti-PD-1, Singapore ASTR anti-PD-1, Sutro anti-PD-1, Rinat anti-PD-1, Biocad anti-PD-1, Enumeral anti-PD-1 or ENUM 388D4, Kadmon anti-PD-1, BMS-936559, avelumab and/or durvalumab or any combination thereof.

6. The composition of claim 1, wherein the KRAS signaling comprises aberrant KRAS signaling.

7. The composition of claim 1, wherein the modulator of KRAS signaling is effective at inhibiting oncogenic KRAS signaling.

8. The composition of claim 1, wherein the modulator of KRAS signaling is ineffective at reducing KRAS induced signaling activity in the absence of oncogenic KRAS gene expression.

9. The composition of claim 1, wherein the modulator of oncogenic KRAS signaling comprises an RNA interfering agent.

10. The composition of claim 9, wherein the RNA interfering agent comprises an KRAS-specific asymmetric interfering RNA (aiRNA).

11. A pharmaceutical composition comprising the composition of claim 1.

12. A method for enhancing an immune response against a tumor in a subject comprising administering to the subject

an effective amount of a modulator of KRAS signaling, and

an effective amount of an immune checkpoint inhibitor,

wherein the combination of the modulator of oncogenic KRAS signaling and the immune checkpoint inhibitor is effective at sensitizing tumor cells in a subject to the immune checkpoint inhibitor.

13. The method of claim 12, wherein sensitizing tumor cells in a subject to the immune checkpoint inhibitor enhances tumor cell-specific T cell cytotoxicity.

14. The method of claim 12, wherein the subject's tumor cells are resistant to treatment with the immune checkpoint inhibitor.

15. The method of claim 12, wherein the immune checkpoint inhibitor is effective at targeting an endogenous immune checkpoint protein or fragment thereof chosen from PD-1, PD-L1, PDL2, CD28, CD80, CD86, CTLA4, B7RP1, ICOS, B7RPI, B7- H3, B7-H4, BTLA, HVEM, KIR, TCR, LAG3, CD 137, CD137L, OX40, OX40L, CD27, CD70, CD40, CD40L, TIM3, GAL9, ADORA, CD276, VTCN1, IDOI, KIR3DL1, HAVCR2, VISTA, and/or CD244 or any combination thereof.

16. The method of claim 12, wherein the immune checkpoint inhibitor is effective at blocking the interaction of programmed cell death protein 1 (PD-1) receptor with programmed cell death 1 ligand 1 (PD-L1).

17. The method of claim 12, wherein the immune checkpoint inhibitor is chosen from ipilimumab, tremelimumab, atezolizumab, nivolumab, pembrolizumab, JS001, REGN2810, SHR-1210, MEDI0680, PDR001, BGB-A317, TSR-042, PF-06801591, Ningbo Cancer Hosp. anti-PD-1 CAR, Medimmune anti-PD-1, Isis anti-PD-1, UCB anti-PD-1 or 948.g1, Dana-Farber anti-PD-1, STI-1110, Suzhou Stainwei Biotech anti-PD-1, Haixi pembrolizumab biosimilar, Livzon anti-PD-1, MabQuest anti-PD-1, Singapore ASTR anti-PD-1, Sutro anti-PD-1, Rinat anti-PD-1, Biocad anti-PD-1, Enumeral anti-PD-1 or ENUM 388D4, Kadmon anti-PD-1, BMS-936559, avelumab and/or durvalumab or any combination thereof.

18. The method of claim 12, wherein the subject's tumor expresses an oncogenic KRAS.

19. The method of claim 18, wherein the oncogenic KRAS comprises a mutation of at least one amino acid residue of SEQ ID NO.: 981.

20. The method of claim 18, wherein the oncogenic KRAS comprises an activating mutation of amino acid residues G12, G13, S17, P34, and/or Q61 of SEQ ID NO.: 981.

21. The method of claim 20, wherein the activating mutation is chosen from G12C, G12S, G12R, G12F, G12L, G12N, G12A, G12D, G12V, G13C, G13S, G13D, G13V, G13P, S17G, P34S, Q61K, Q61L, Q61R, and/or Q61H.

22. The method of claim 12, wherein the modulator of KRAS signaling inhibits oncogenic KRAS signaling.

23. The method of claim 22, wherein the oncogenic KRAS signaling comprises signaling by the RAS/RAF/MEK/ERK/FRA-1 pathway.

24. The method of claim 12, wherein the modulator of KRAS signaling is ineffective at reducing KRAS induced signaling activity in the absence of oncogenic KRAS gene expression.

25. The method of claim 12, wherein the modulator of oncogenic KRAS signaling inhibits RAS/RAF/MEK/ERK/FRA-1 signaling.

26. The method of claim 25, wherein the modulator of oncogenic KRAS signaling comprises a small molecule inhibitor of a member of the RAS/RAF/MEK/ERK/FRA-1 pathway.

27. The method of claim 12, wherein the modulator of KRAS signaling comprises a KRAS-specific RNA interfering agent.

28. The method of claim 27, wherein the RNA interfering agent is specific for the expressed oncogenic KRAS.

29. The method of claim 27, wherein the RNA interfering agent comprises a KRAS-specific small interfering RNA (siRNA), small hairpin RNA (shRNA), microRNA (miRNA), or a piwiRNA (piRNA).

30. The method of claim 27, wherein the RNA interfering agent comprises a KRAS-specific asymmetric interfering RNA (aiRNA).

31. The method of claim 30, wherein the KRAS-specific asymmetric interfering RNA (aiRNA) is effective at inhibiting KRAS mRNA levels by at least 95%.

32. The method of claim 30, wherein the KRAS-specific asymmetric interfering RNA (aiRNA) is specific for the expressed oncogenic KRAS.

33. The method of claim 12, wherein the modulator of KRAS signaling is effective at reducing endogenous FRA-1 DNA binding activity in the subject's tumor cells.

34. The method of claim 12, wherein the modulator of KRAS signaling is effective at inhibiting the expression of an immune checkpoint molecule in the subject's tumor cells.

35. The method of claim 34, wherein the immune checkpoint molecule is the programmed cell death 1 ligand 1 (PD-L1).

36. The method of claim 12, wherein the tumor is caused by a cancer chosen from pancreatic ductal adenocarcinoma (PDAC), colorectal cancer or non-small-cell lung cancer (NSCLC).

37. The method of claim 36, wherein the cancer is a metastatic cancer, a cancer that is refractory to chemotherapy, a cancer that is refractory to radiotherapy and/or a cancer that has relapsed.

38. The method of claim 12, wherein the modulator of KRAS signaling is ineffective at reducing KRAS induced signaling activity in the absence of oncogenic KRAS gene expression.

39. A modulator of oncogenic KRAS signaling.

40. The modulator of claim 39, wherein the KRAS signaling comprises aberrant KRAS signaling.

41. The modulator of claim 39, wherein the modulator of KRAS signaling is effective at inhibiting oncogenic KRAS signaling.

42. The modulator of claim 39, wherein the modulator of KRAS signaling is ineffective at reducing KRAS induced signaling activity in the absence of oncogenic KRAS gene expression.

43. The modulator of claim 39, comprising an RNA interfering agent.

44. The modulator of claim 39, comprising an asymmetric interfering RNA (aiRNA).

45. The modulator of claim 44, wherein the asymmetric interfering RNA comprises a sense strand sequence that is at least 50% identical to a sequence chosen from SEQ ID NOs: 320-637.

46. The modulator of claim 44, wherein the asymmetric interfering RNA comprises a sense strand sequence chosen from SEQ ID NOs: 320-637.

47. The modulator of claim 44, wherein the asymmetric interfering RNA comprises an antisense strand sequence that is at least 50% identical to a sequence chosen from SEQ ID NOs: 638-955.

48. The modulator of claim 44, wherein the asymmetric interfering RNA comprises an antisense strand sequence chosen from SEQ ID NOs: 638-955.

49. A composition comprising an effective amount of a modulator of oncogenic KRAS signaling of any one of claims 39-48.

50. A method for changing the efficacy or/and safety of a therapeutic agent comprising administering an effective amount of a modulator of KRAS signaling.

51. A method for changing the efficacy or/and safety of a therapeutic agent comprising administering an effective amount of an asymmetric interfering RNA (aiRNA).

52. A method for changing the efficacy or/and safety of a therapeutic agent comprising administering an effective amount of an asymmetric interfering RNA (aiRNA) comprising a sense strand sequence chosen from SEQ ID NOs: 320-637.

53. A method for changing the efficacy or/and safety of a therapeutic agent comprising administering an effective amount of an asymmetric interfering RNA (aiRNA) comprising antisense strand sequence chosen from SEQ ID NOs: 638-955.

54. The method of any one of claims 50-53, wherein the efficacy of the therapeutic agent is enhanced.

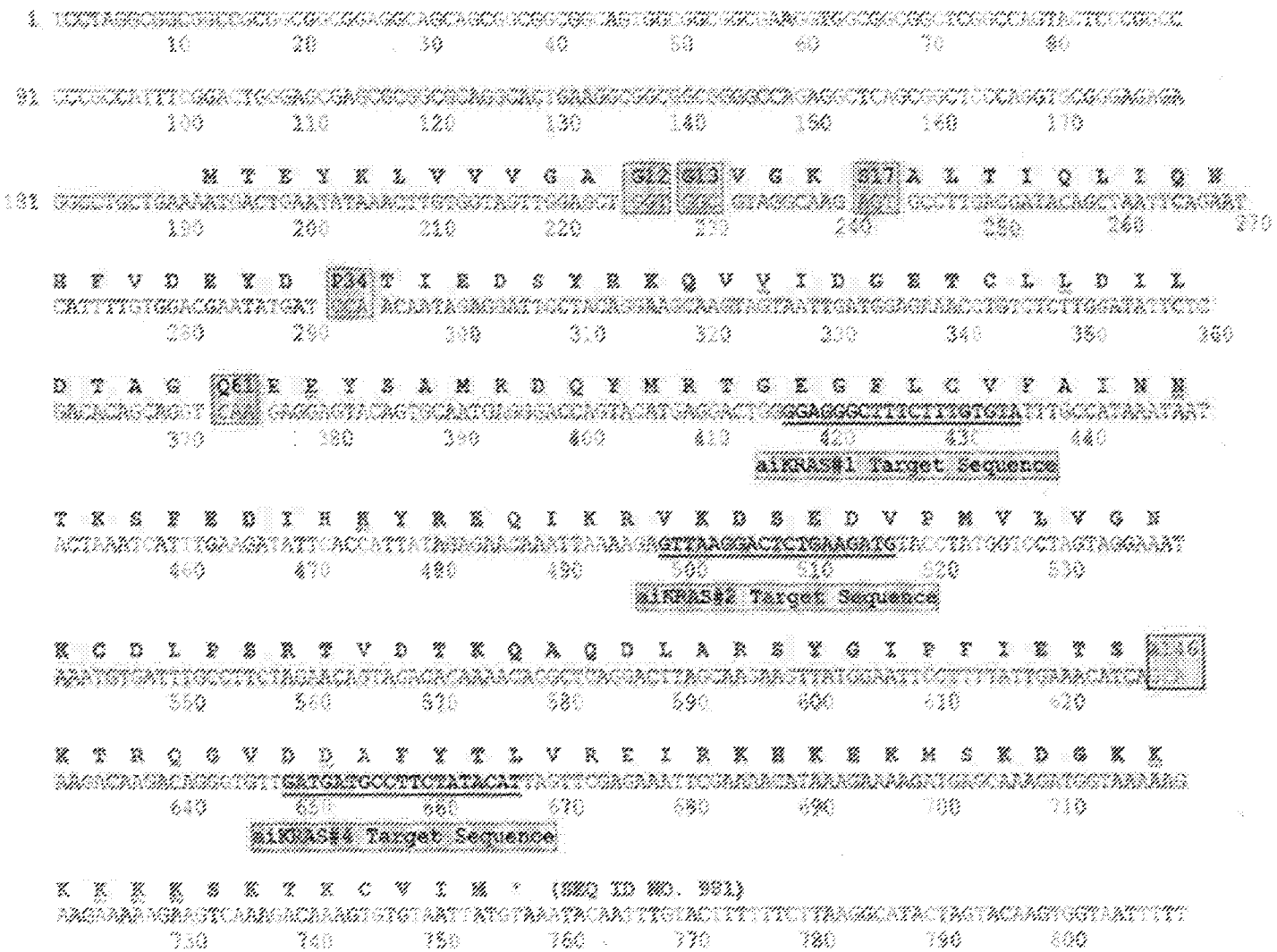
55. The method of any one of claims 50-54, wherein the safety of the therapeutic agent is enhanced.

56. The method of any one of claims 50-55, wherein the therapeutic agent is an immune checkpoint inhibitor.

57. The method of any one of claims 50-56, wherein the therapeutic agent is chosen from ipilimumab, tremelimumab, atezolizumab, nivolumab, pembrolizumab, JS001, REGN2810, SHR-1210, MEDI0680, PDR001, BGB-A317, TSR-042, PF-06801591, Ningbo Cancer Hosp. anti-PD-1 CAR, Medimmune anti-PD-1, Isis anti-PD-1, UCB anti-PD-1 or 948.g1, Dana-Farber anti-PD-1, STI-1110, Suzhou Stainwei Biotech anti-PD-1, Haixi pembrolizumab biosimilar, Livzon anti-PD-1, MabQuest anti-PD-1, Singapore ASTR anti-PD-1, Sutro anti-PD-1, Rinat anti-PD-1, Biocad anti-PD-1, Enumeral anti-PD-1 or ENUM 388D4, Kadmon anti-PD-1, BMS-936559, avelumab and/or durvalumab or any combination thereof.

	TARGET SEQUENCES	SENSE STRANDS 5'-	ANTI-SENSE STRANDS 5'-
aiControl (Scramble)	CAGGTAGTTATAGTCGATG (SEQ ID NO.: 956)	GUAGUUAUAGUCGAU (SEQ ID NO.: 957)	AACAUCGACUAUAACUACCUG (SEQ ID NO.: 958)
aiKRAS#1	GGAGGGCTTTCTTTGTGTA (SEQ ID NO.: 959)	GGGCUUUCUUUGUGU (SEQ ID NO.: 960)	AAUACACAAAGAAAGCCCUCC (SEQ ID NO.: 961)
aiKRAS#2	GTTAAGGACTCTGAAGATG (SEQ ID NO.: 962)	AAGGACUCUGAAGAU (SEQ ID NO.: 963)	AACAUCUUCAGAGUCCUUAAC (SEQ ID NO.: 964)
aiKRAS#3	CAGTTGATTACTTCTTATT (SEQ ID NO.: 965)	UUGAUUACUUCUUAU (SEQ ID NO.: 966)	AAAAUAAGAAGUAAUCAACUG (SEQ ID NO.: 967)
aiKRAS#4	GATGATGCCTTCTATACAT (SEQ ID NO.: 968)	GAUGCCUUCUAUACA (SEQ ID NO.: 969)	AAAUGUAUAGAAGGCAUCAUC (SEQ ID NO.: 970)
aiPD-L1	GGTTGTGAATGATTTCTTT (SEQ ID NO.: 971)	UGUGAAUGAUUUCUU (SEQ ID NO.: 972)	AAAAAGAAAUCAUUCACAACC (SEQ ID NO.: 973)
aiFra-1#1	CCTCTAATGAGACTGACCA (SEQ ID NO.: 974)	CUAAUGAGACUGACC (SEQ ID NO.: 975)	AAUGGUCAGUCUCAUUAGAGG (SEQ ID NO.: 976)
aiFra-1#2	CCAGAGACTTTGTAGATCC (SEQ ID NO.: 977)	GAGACUUUGUAGAUC (SEQ ID NO.: 978)	AAGGAUCUACAAAGUCUCUGG (SEQ ID NO.: 979)

FIG. 1A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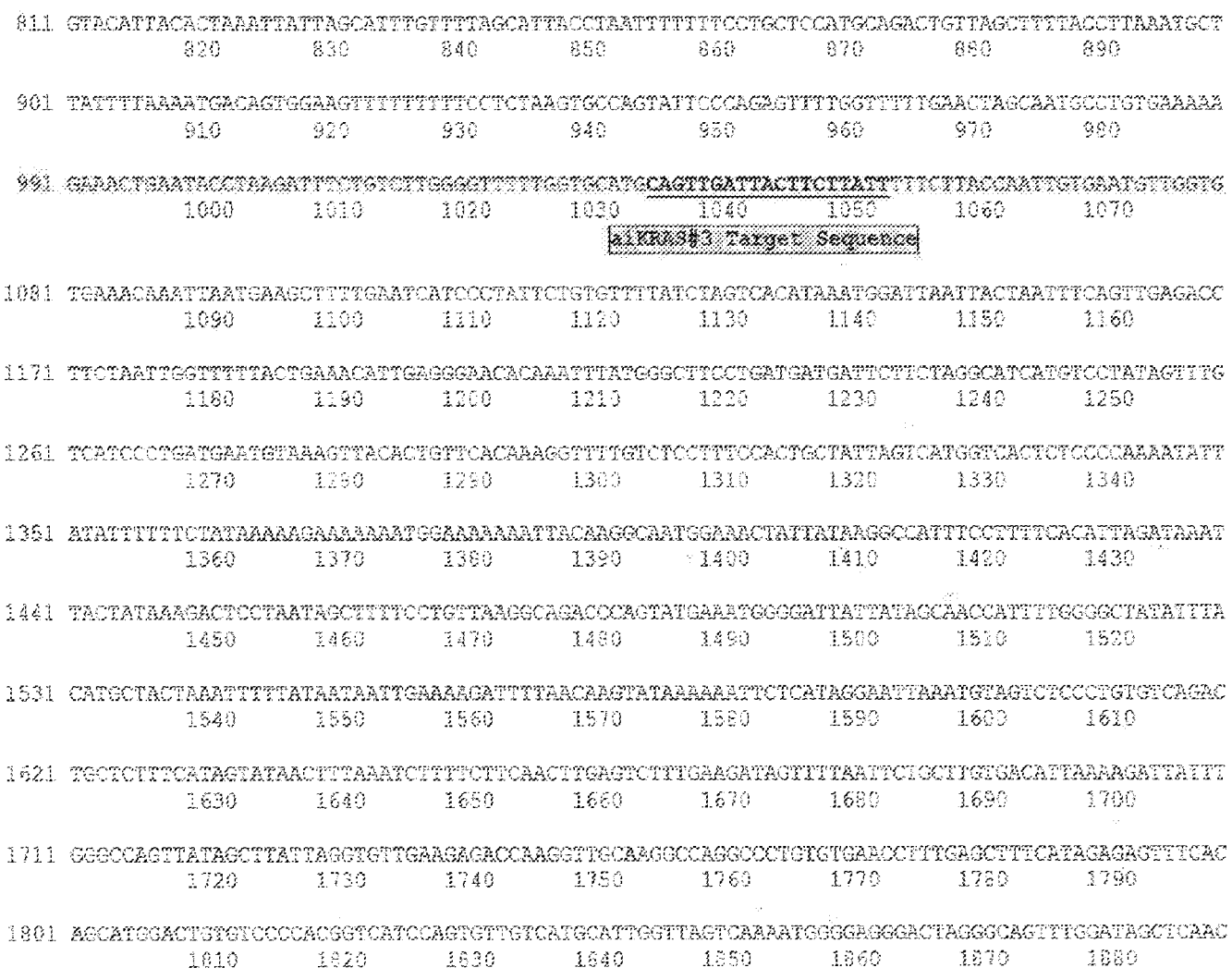
aiRNA#4 Target Sequence
aiRNA#6 Target Sequence


811 GTACATTACACTAAATTATTAGCATTTGTTTTAGCATTACCTAATTTTTTTCCTGCTCCATGCAGACTGTTAGCTTTTACCTTAAATGCT
820 830 840 850 860 870 880 890
901 TATTTTAAAATGACAGTGGAAGTTTTTTTTTCCTCTAAGTGCCAGTATTCCCAGAGTTTTGGTTTTTGAACTAGCAATGCCTGTGAAAAA
910 920 930 940 950 960 970 980
991 GAAACTGAATACCTAAGATTTCTGTCTTGGGCTTTTTGGTGCATGCAGTTGATTACTTCTTATTTTTCTTACCAATTGTGAATGTTGGTG
1000 1010 1020 1030 1040 1050 1060 1070
siRNA#3 Target Sequence
1081 TGAAACAAATTAATGAAGCTTTTGAATCATCCCTATTCTGTGTTTTATCTAGTCACATAAATGGATTAATTACTAATTTCAGTTGAGACC
1090 1100 1110 1120 1130 1140 1150 1160
1171 TTCTAATTGGTTTTTACTGAAACATTGAGGGAACACAAATTTATGGGCTTCCTGATGATGATTCTTCTAGGCATCATGTCCTATAGTTTG
1180 1190 1200 1210 1220 1230 1240 1250
1261 TCATCCCTGATGAATGTAAAGTTACACTGTTCACAAAGGTTTTGTCTCCTTTCCACTGCTATTAGTCATGGTCACTCTCCCCAAAATATT
1270 1280 1290 1300 1310 1320 1330 1340
1351 ATATTTTTTCTATAAAAAGAAAAAAAATGGAAAAAAATTACAAGGCAATGGAAACTATTATAAGGCCATTTCCTTTTCACATTAGATAAAT
1360 1370 1380 1390 1400 1410 1420 1430
1441 TACTATAAAGACTCCTAATAGCTTTTCCTGTTAAGGCAGACCCAGTATGAAATGGGGATTATTATAGCAACCATTTTGGGGCTATATTTA
1450 1460 1470 1480 1490 1500 1510 1520
1531 CATGCTACTAAATTTTTATAATAATTGAAAACATTTTAACAAGTATAAAAAATTCTCATAGGAATTAAATGTAGTCTCCCTGTGTCAGAC
1540 1550 1560 1570 1580 1590 1600 1610
1621 TGCTCTTTCATAGTATAACTTTAAATCTTTTCTTCAACTTGAGTCTTTGAAGATAGTTTTAATTCTGCTTGTGACATTAAAAGATTATTT
1630 1640 1650 1660 1670 1680 1690 1700
1711 GGGCCAGTTATAGCTTATTAGGTGTTGAAGAGACCAAGGTTGCAAGGCCAGGCCCTGTGTGAACCTTTGAGCTTTCATAGAGAGTTTCAC
1720 1730 1740 1750 1760 1770 1780 1790
1801 AGCATGGACTGTGTCCCCACGGTCATCCAGTGTTGTCATGCATTGGTTAGTCAAAATGGGGAGGGACTAGGGCAGTTTGGATAGCTCAAC
1810 1820 1830 1840 1850 1860 1870 1880

1891 AAGATACAATCTCACTCTGTGGTGGTCCTGCTGACAAATCAAGAGCATTGCTTTTCTTTCTTAAGAAAACAAACTCTTTTTTAAAAATTA
1900 1910 1920 1930 1940 1950 1960 1970

1981 CTTTTAAATATTAACTCAAAAGTTGAGATTTTGGGGTGGTGGTGTGCCAAGACATTAATTTTTTTTTTAAACAATGAAGTGAAAAAGTTT
1990 2000 2010 2020 2030 2040 2050 2060

2071 TACAATCTCTAGGTTTGGCTAGTTCTCTTAACACTGGTTAAATTAACATTGCATAAACACTTTTCAAGTCTGATCCATATTTAATAATGC
2080 2090 2100 2110 2120 2130 2140 2150

2161 TTTAAAATAAAAATAAAAACAATCTTTTGATAAATTTAAAATGTTACTTATTTTAAAATAAATGAAGTGAGATGGCATGGTGAGGTGAA
2170 2180 2190 2200 2210 2220 2230 2240

2251 AGTATCACTGGACTAGGAAGAAGGTGACTTAGGTTCTAGATAGGTGTCTTTTAGGACTCTGATTTTGAGGACATCACTTACTATCCATTT
2260 2270 2280 2290 2300 2310 2320 2330

2341 CTTCATGTTAAAAGAAGTCATCTCAAACTCTTAGTTTTTTTTTTTTACAACTATGTAATTTATATTCCATTTACATAAGGATACACTTAT
2350 2360 2370 2380 2390 2400 2410 2420

2431 TTGTCAAGCTCAGCACAATCTGTAAATTTTTAACCTATGTTACACCATCTTCAGTGCCAGTCTTGGGCAAAATTGTGCAAGAGGTGAAGT
2440 2450 2460 2470 2480 2490 2500 2510

2521 TTATATTTGAATATCCATTCTCGTTTTAGGACTCTTCTTCCATATTAGTGTCATCTTGCCTCCCTACCTTCCACATGCCCCATGACTTGA
2530 2540 2550 2560 2570 2580 2590 2600

2611 TGCAGTTTTAAATACTTGTAATTCCCCTAACCATAAGATTTACTGCTGCTGTGGATATCTCCATGAAGTTTTCCCACTGAGTCACATCAGA
2620 2630 2640 2650 2660 2670 2680 2690

2701 AATGCCCTACATCTTATTTCCTCAGGGCTCAAGAGAATCTGACAGATACCATAAAGGGATTTGACCTAATCACTAATTTTCAGGTGGTGG
2710 2720 2730 2740 2750 2760 2770 2780

2791 CTGATGCTTTGAACATCTCTTTGCTGCCCAATCCATTAGCGACAGTAGGATTTTTCAAACCTGGTATGAATAGACAGAACCCTATCCAGT
2800 2810 2820 2830 2840 2850 2860 2870

```
2881 GGAAGGAGAATTTAATAAAGATAGTGCTGAAAGAATTCCTTAGGTAATCTATAACTAGGACTACTCCTGGTAACAGTAATACATTCCATT
          2890       2900       2910       2920       2930       2940       2950       2960

2971 GTTTTACTAACCACAAAATCTTCATGCAATGAAAAATACTTTAATTCATGAAGCTTACTTTTTTTTTTTGGTGTCAGACTCTCGCTCTTGT
          2980       2990       3000       3010       3020       3030       3040       3050

3061 CACCCAGGCTGGAATGCAGTGGCGCCATCTCAGCTCACTGCAACCTCCATCTCCCAGGTTCAAGCGATTCTCGTGCCTCGGCCTCCTGAG
          3070       3080       3090       3100       3110       3120       3130       3140

3141 TAGCTGGGATTACAGGCGTGTGCCACTACACTCAACTAATTTTTGTATTTTTAGGAGACGGGGTTTCACCCTGTTGGCCAGGCTGGTC
          3150       3160       3170       3180       3190       3200       3210       3220

3221 TCGAACTCCTGACCTCAAGTGATTCACCCACCTTGGCCTCATAAACCTGTTTTGCAGAACTCATTTATTCAGCAAATATTTATTGAGTGC
          3230       3240       3250       3260       3270       3280       3290       3300

3301 CTACCAGATGCCAGTCACCGCACAAGGCACTGGCTATATGGTATCCCCAAACAAGAGACATAATCCCGGTCCTTAGGTAGTGCTAGTGTG
          3310       3320       3330       3340       3350       3360       3370       3380

3381 GTCTGTAATATCTTACTAAGGCCTTTGGTATACGACCCAGAGATAACACGATGCGTATTTTAGTTTTGCAAAGAAGGGGTTTGGTCTCTG
          3390       3400       3410       3420       3430       3440       3450       3460

3461 TGCCAGCTCTATAATTGTTTTGCTACGATCCACTGAAACTCTTCGATCAAGCTACTTTATGTAAATCACTTCATTGTTTTAAAGGAATA
          3470       3480       3490       3500       3510       3520       3530       3540

3541 AACTTGATTATATTGTTTTTTTTATTTGGCATAACTGTGATTCTTTTAGGACAATTACTGTACACATTAAGGTGTATGTCAGATATTCATA
          3550       3560       3570       3580       3590       3600       3610       3620

3621 TTGACCCAAATGTGTAATATTCCAGTTTTCTCTGCATAAGTAACTAAAATATACTTAAAAATTAATAAGTTTTATCTGGGTACAAATAAAC
          3630       3640       3650       3660       3670       3680       3690       3700

3701 AGGTGCCTGAACTAGTTCACAGACAAGGAAACTTCTATGTAAAAATCACTATGATTTCTGAATTGCTATGTGAAACTACAGATCTTTGGA
          3710       3720       3730       3740       3750       3760       3770       3780

3781 ACACTGTTTAGGTAGGGTGTTAAGACTTACACAGTACCTCGTTTCTACACAGAGAAGAAATGGCCATACTTCAGGAACTGCAGTGCTTA
          3790       3800       3810       3820       3830       3840       3850       3860

3861 TCAGGGGATATTTAGGCCTCTTGAATTTTTGATGTAGATGGCATTTTTTTAAGGTAGTGGTTAATTACCTTTATGTGAACTTTGAATGG
          3870       3880       3890       3900       3910       3920       3930       3940
```

4051 TTTAACAAAAGATTTGTTTTTGTAGAGATTTTAAAGGGGGAGAATTCTAGAAATAAATGTTACCTAATTATTACAGCCTTAAAGACAAAA
4060 4070 4080 4090 4100 4110 4120 4130

4141 ATCCTTGTTGAAGTTTTTTTAAAAAAAGCTAAATTACATAGACTTAGGCATTAACATGTTTGTGGAAGAATATAGCAGACGTATATTGTA
4150 4160 4170 4180 4190 4200 4210 4220

4231 TCATTTGAGTGAATGTTCCCAAGTAGGCATTCTAGGCTCTATTTAACTGAGTCACACTGCATAGGAATTTAGAACCTAACTTTTATAGGT
4240 4250 4260 4270 4280 4290 4300 4310

4321 TATCAAAACTGTTGTCACCATTGCACAATTTTGTCCTAATATATACATAGAAACTTTGTGGGGCATGTTAAGTTACAGTTTGCACAAGTT
4330 4340 4350 4360 4370 4380 4390 4400

4411 CATCTCATTTGTATTCCATTGATTTTTTTTTTCTTCTAAACATTTTTTCTTCAAACAGTATATAACTTTTTTTAGGGGATTTTTTTTTAG
4420 4430 4440 4450 4460 4470 4480 4490

4501 ACAGCAAAAACTATCTGAAGATTTCCATTTGTCAAAAAGTAATGATTTCTTGATAATTGTGTAGTAATGTTTTTTAGAACCCAGCTGTTA
4510 4520 4530 4540 4550 4560 4570 4580

4591 CCTTAAAGCTGAATTTATATTTAGTAACTTCTGTGTTAATACTGGATAGCATGAATTCTGCATTGAGAAACTGAATAGCTGTCATAAAAT
4600 4610 4620 4630 4640 4650 4660 4670

4681 GAAACTTTCTTTCTAAAGAAAGATACTCACATGAGTTCTTGAAGAATAGTCATAACTAGATTAAGATCTGTGTTTTAGTTTAATAGTTTG
4690 4700 4710 4720 4730 4740 4750 4760

4771 AAGTGCCTGTTTGGGATAATGATAGGTAATTTAGATGAATTTAGGGGAAAAAAAAAGTTATCTGCAGATATGTTGAGGGCCCATCTCTCCC
4780 4790 4800 4810 4820 4830 4840 4850

4861 CCCACACCCCCACAGAGCTAACTGGGTTACAGTGTTTTATCCGAAAGTTTCCAATTCCACTGTCTTGTGTTTTCATGTTGAAAATACTTT
4870 4880 4890 4900 4910 4920 4930 4940

4951 TGCATTTTTCCTTTGAGTGCCAATTTCTTACTAGTACTATTTCTTAATGTAACATGTTTACCTGGAATGTATTTAACTATTTTTGTATA
4960 4970 4980 4990 5000 5010 5020 5030

5041 GTGTAAACTGAAACATGCACATTTTGTACATTGTGCTTTCTTTTGTGGGACATATGCAGTGTGATCCAGTTGTTTTCCATCATTTGGTTG
5050 5060 5070 5080 5090 5100 5110 5120

5131 CGCTGACCTAGGAATGTTGGTCATATCAAACATTAAAAATGACCACTCTTTTAATTGAAATTAACTTTTAAATGTTTATAGGAGTATGTG
5140 5150 5160 5170 5180 5190 5200 5210

5221 CTGTGAAGTGATCTAAAATTTGTAATATTTTTGTCATGAACTGTACTACTCCTAATTATTGTAATGTAATAAAAATAGTTACAGTGACTA
5230 5240 5250 5260 5270 5280 5290 5300

5311 TGAGTGTGTATTATTCATGAAATTTGAACTGTTTGCCCCGAAATGGATATGGAATACTTTRTAAGCCATAGACACTATAGTATRCCAGT
5320 5330 5340 5350 5360 5370 5380 5390

5401 GAATCTTTTATGCAGCTTGTTAGRAGTATCCTTTATTTCTAAAAGGTGCTGTGGATATTATGTAAAGGCGTGTTTGCTTAAACTTAAAAC
5410 5420 5430 5440 5450 5460 5470 5480

5491 CATATTTAGAAGTAGATGCAAAACAAATCTGCCTTTATGACAAAAAAATAGGATAACATTATTTATTTATTCCTTTTATCAAAGAAGGT
5500 5510 5520 5530 5540 5550 5560 5570

5581 AATTGATACACAACAGGTGACTTGGTTTTAGGCCCAAAGGTAGCAGCAGCAACATTAATAATGGAAATAATTGAATAGTTAGTTATGTAT
5590 5600 5610 5620 5630 5640 5650 5660

5671 GTTAATGCCAGTCACCAGCAGGCTATTTCAAGGTCAGAAGTAATGACTCCATACATATTATTTATTTCTATAACTACATTTAAATCATTA
5680 5690 5700 5710 5720 5730 5740 5750

5761 CCAGG (SEQ ID NO.: 980)

FIG. 1B

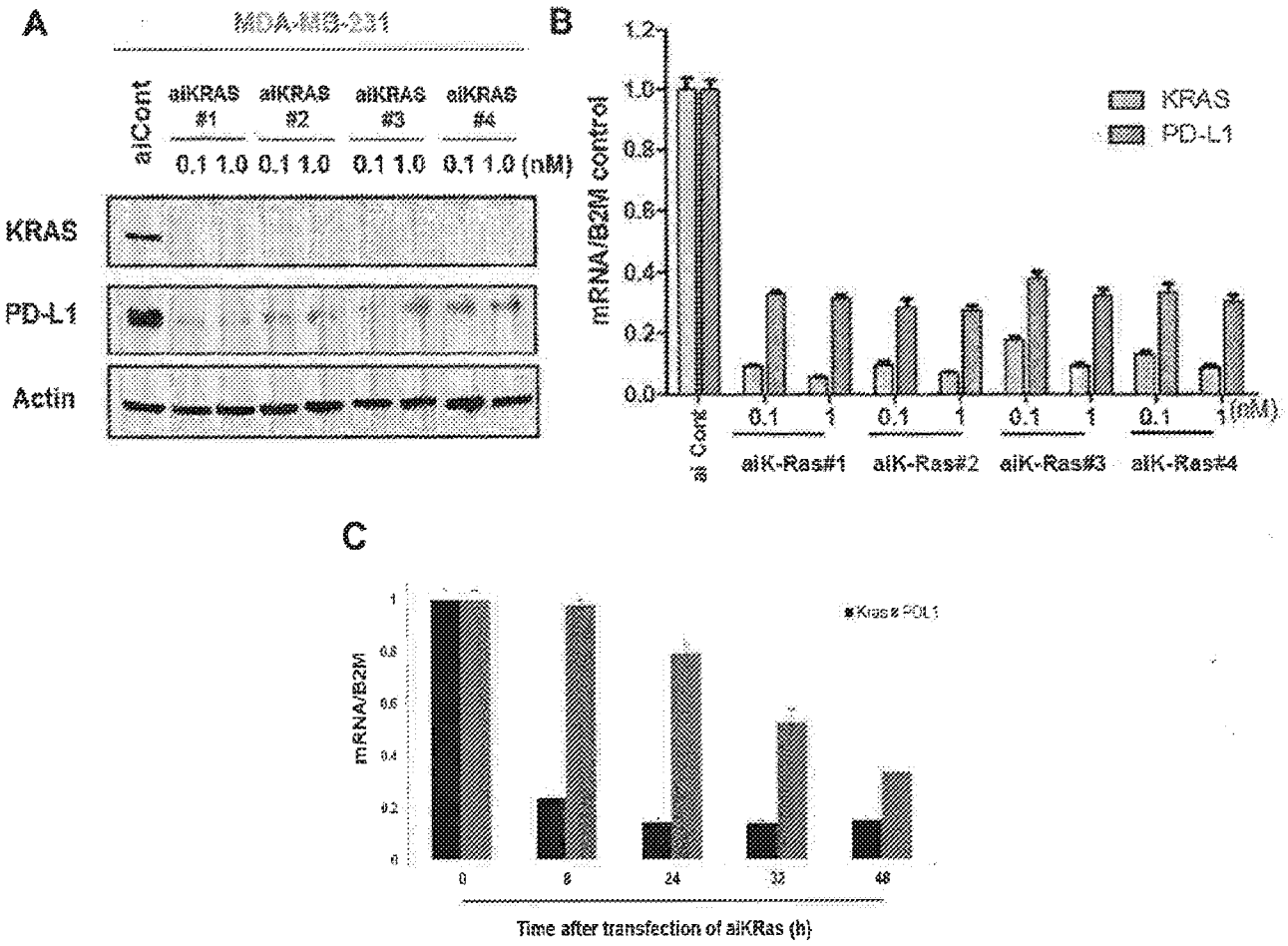

A
MDA-MB-231
aiCont
aiKRAS #1
aiKRAS #2
aiKRAS #3
aiKRAS #4
0.1 1.0 0.1 1.0 0.1 1.0 0.1 1.0 (nM)
KRAS
PD-L1
Actin
B
mRNA/B2M control
KRAS
PD-L1
ai Cont
0.1
1
aiK-Ras#1
aiK-Ras#2
aiK-Ras#3
aiK-Ras#4
(nM)
C
mRNA/B2M
0
8
24
32
48
Time after transfection of aiKRas (h)

FIG. 2

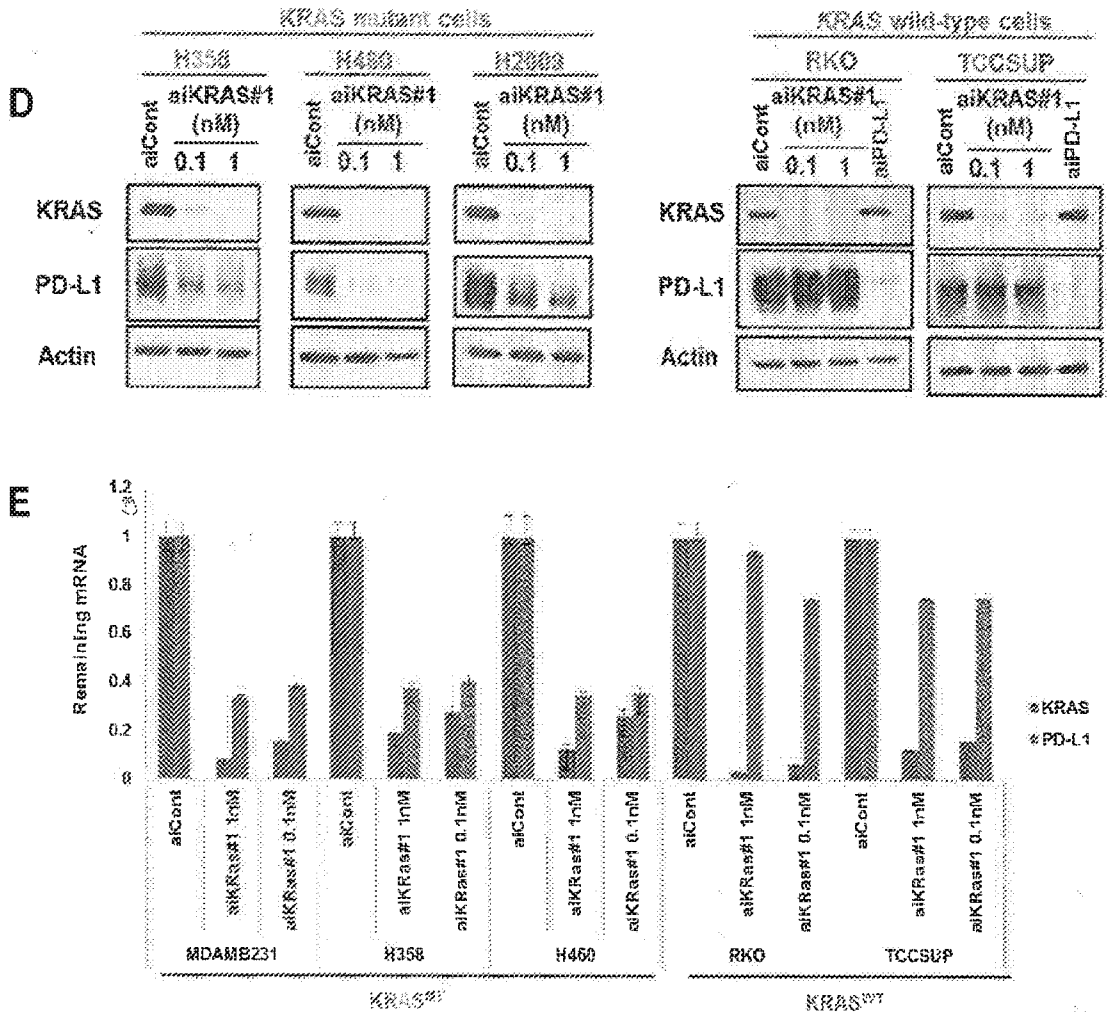

D
KRAS mutant cells
KRAS wild-type cells
H358
H460
H2009
RKO
TCCSUP
siCont
siKRAS#1 (nM)
0.1 1
siPD-L1
KRAS
PD-L1
Actin
E
Remaining mRNA
1.2
1
0.8
0.6
0.4
0.2
0
siCont
siKRas#1 1nM
siKRas#1 0.1nM
MDAMB231
H358
H460
RKO
TCCSUP
KRASMT
KRASWT
KRAS
PD-L1

FIG. 2 (ctd.)

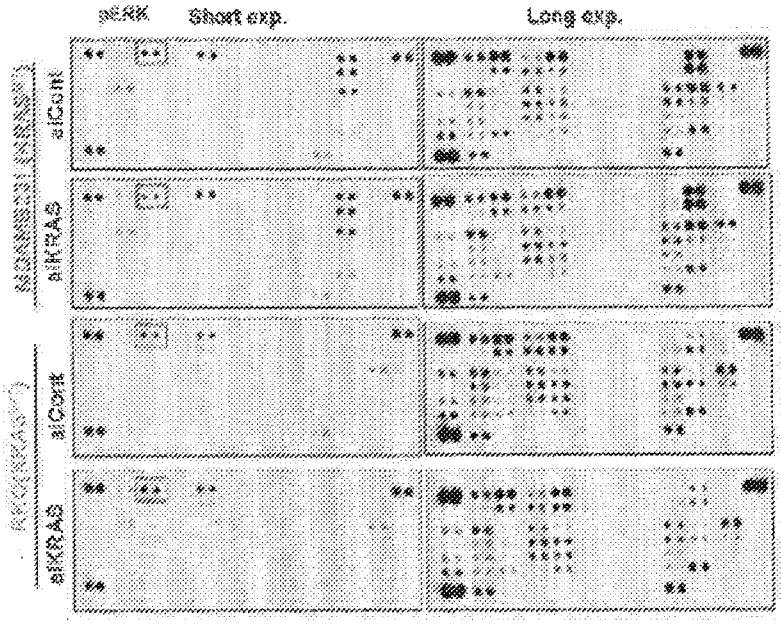

pERK
Short exp.
Long exp.
aiCont
aiKRAS
aiCont
aiKRAS

FIG. 3A

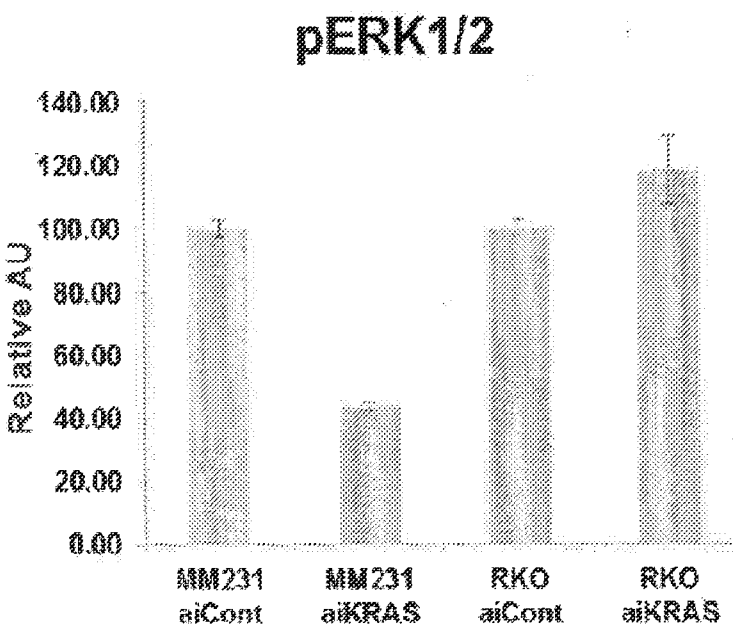

pERK1/2
Relative AU
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
MM231 aiCont
MM231 aiKRAS
RKO aiCont
RKO aiKRAS

FIG. 3B

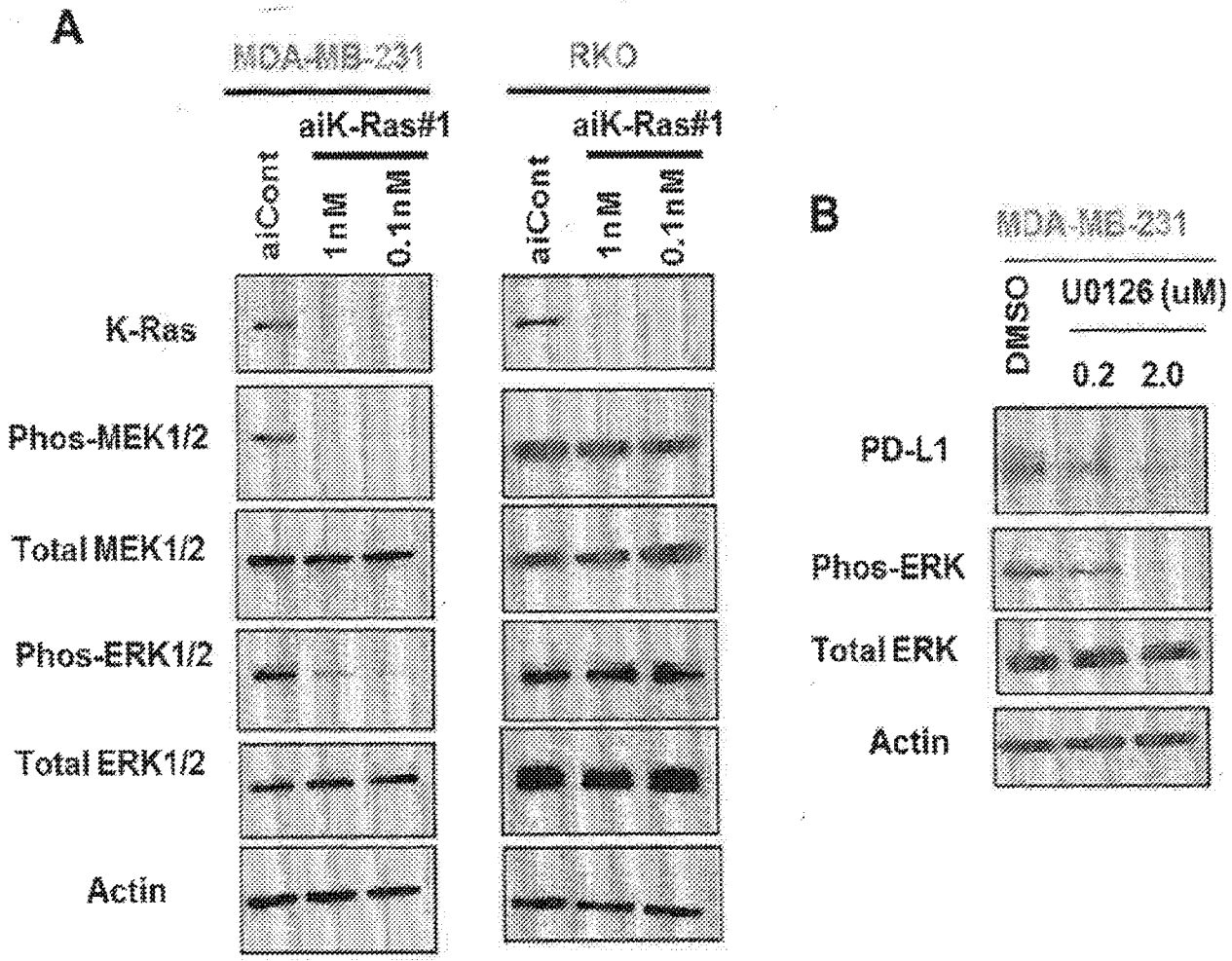

A
MDA-MB-231
RKO
aiK-Ras#1
aiK-Ras#1
aiCont
1nM
0.1nM
aiCont
1nM
0.1nM
K-Ras
Phos-MEK1/2
Total MEK1/2
Phos-ERK1/2
Total ERK1/2
Actin
B
MDA-MB-231
DMSO
U0126 (uM)
0.2
2.0
PD-L1
Phos-ERK
Total ERK
Actin

FIG. 4

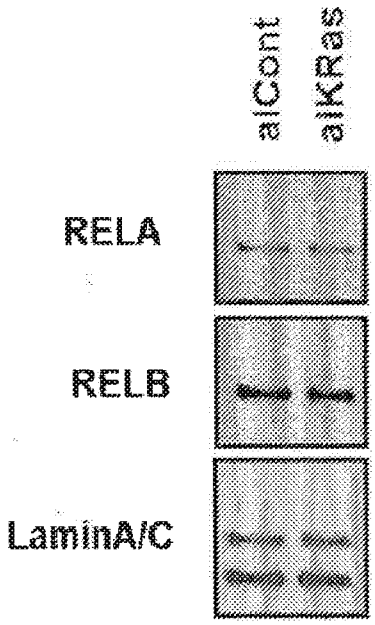

aiCont
aiKRas
RELA
RELB
LaminA/C

FIG. 4C

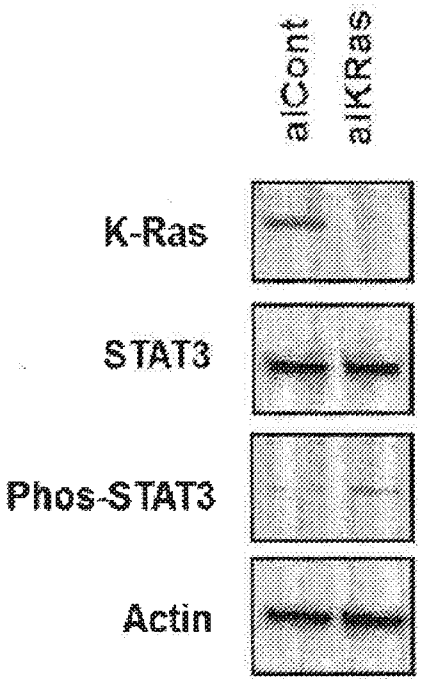

aiCont
aiKRas
K-Ras
STAT3
Phos-STAT3
Actin

FIG. 4D

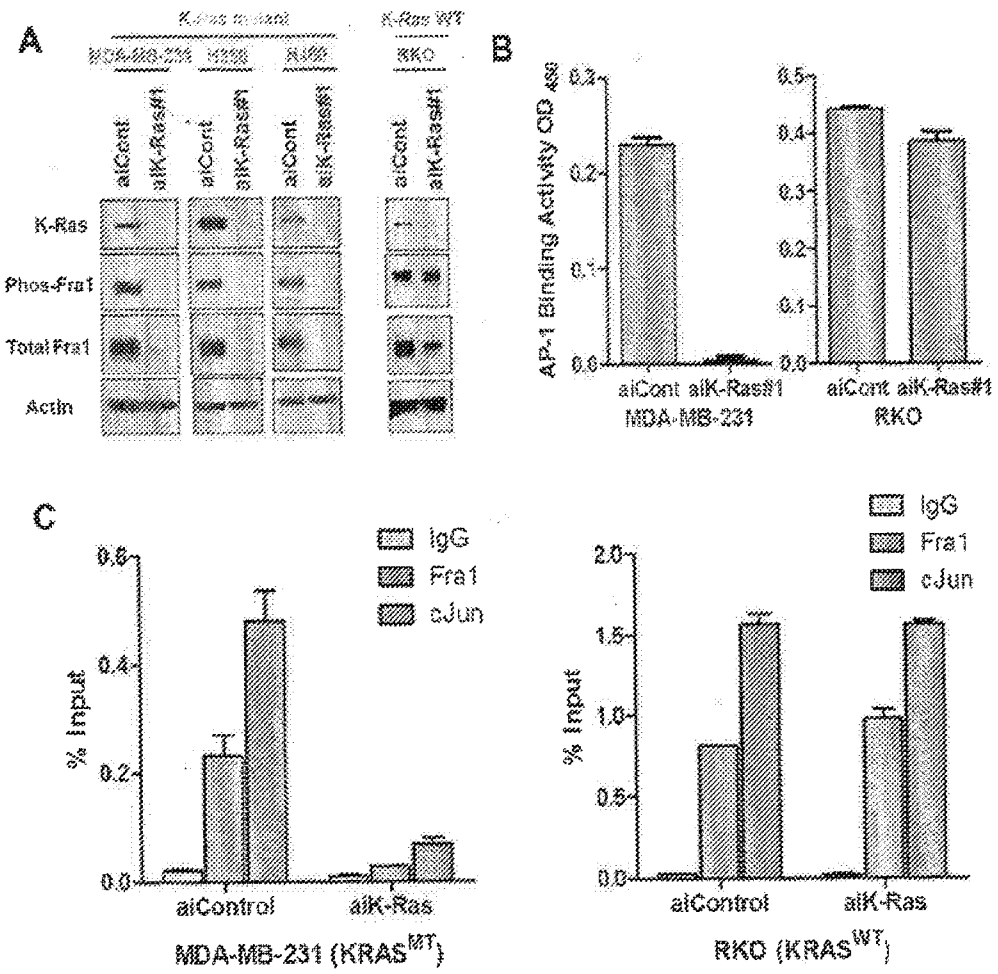

A
K-Ras mutant
K-Ras WT
MDA-MB-231
H358
A549
RKO
aiCont
aiK-Ras#1
K-Ras
Phos-Fra1
Total Fra1
Actin
B
AP-1 Binding Activity OD 450
aiCont aiK-Ras#1
MDA-MB-231
RKO
C
IgG
Fra1
cJun
% Input
aiControl
aiK-Ras
MDA-MB-231 (KRAS^MT)
RKO (KRAS^WT)

FIG. 5

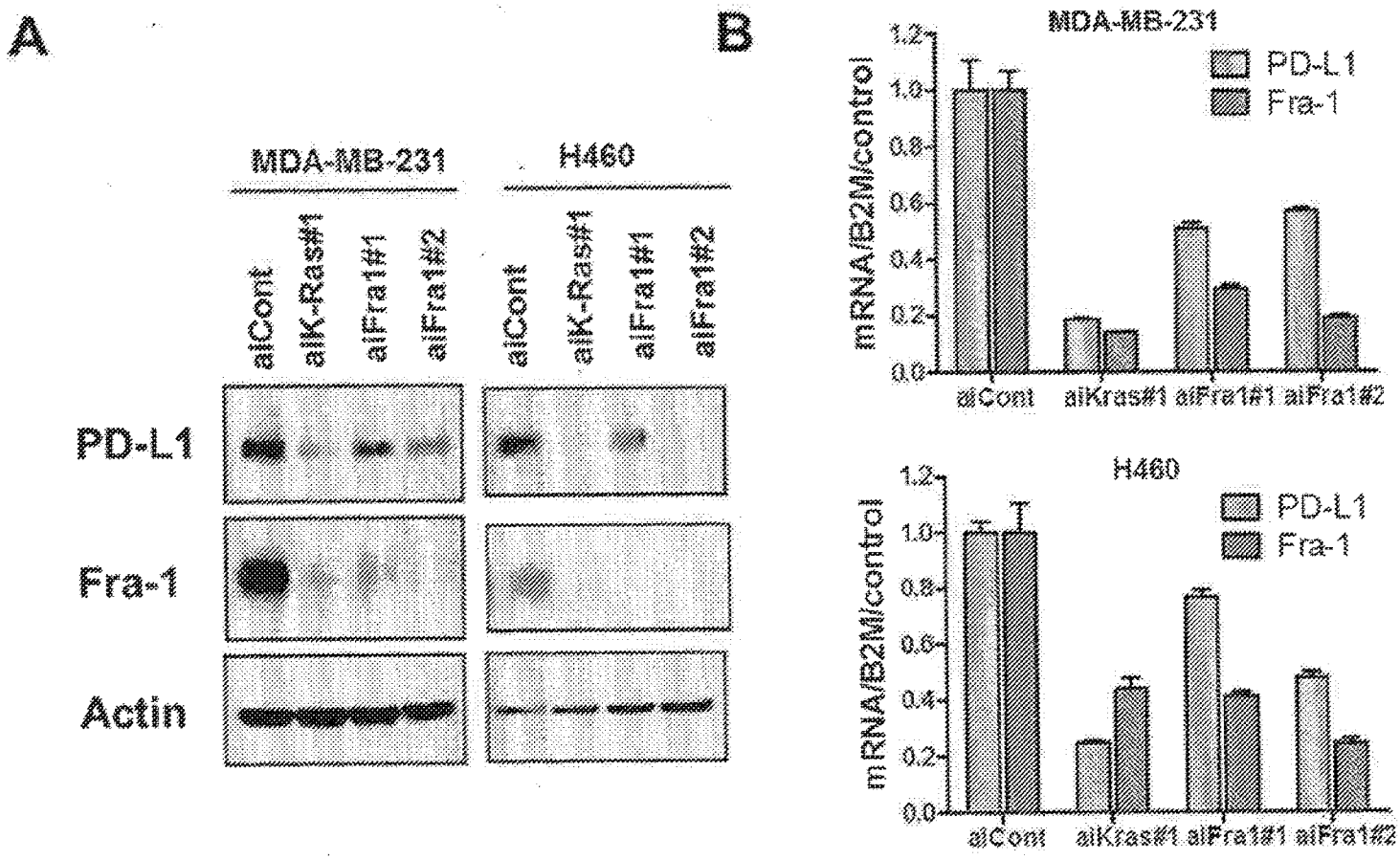

A
B
MDA-MB-231
H460
aiCont
aiK-Ras#1
aiFra1#1
aiFra1#2
PD-L1
Fra-1
Actin
MDA-MB-231
PD-L1
Fra-1
mRNA/B2M/control
1.2
1.0
0.8
0.6
0.4
0.2
0.0
aiCont
aiKras#1
aiFra1#1
aiFra1#2
H460
PD-L1
Fra-1
mRNA/B2M/control
aiCont
aiKras#1
aiFra1#1
aiFra1#2

FIG. 6

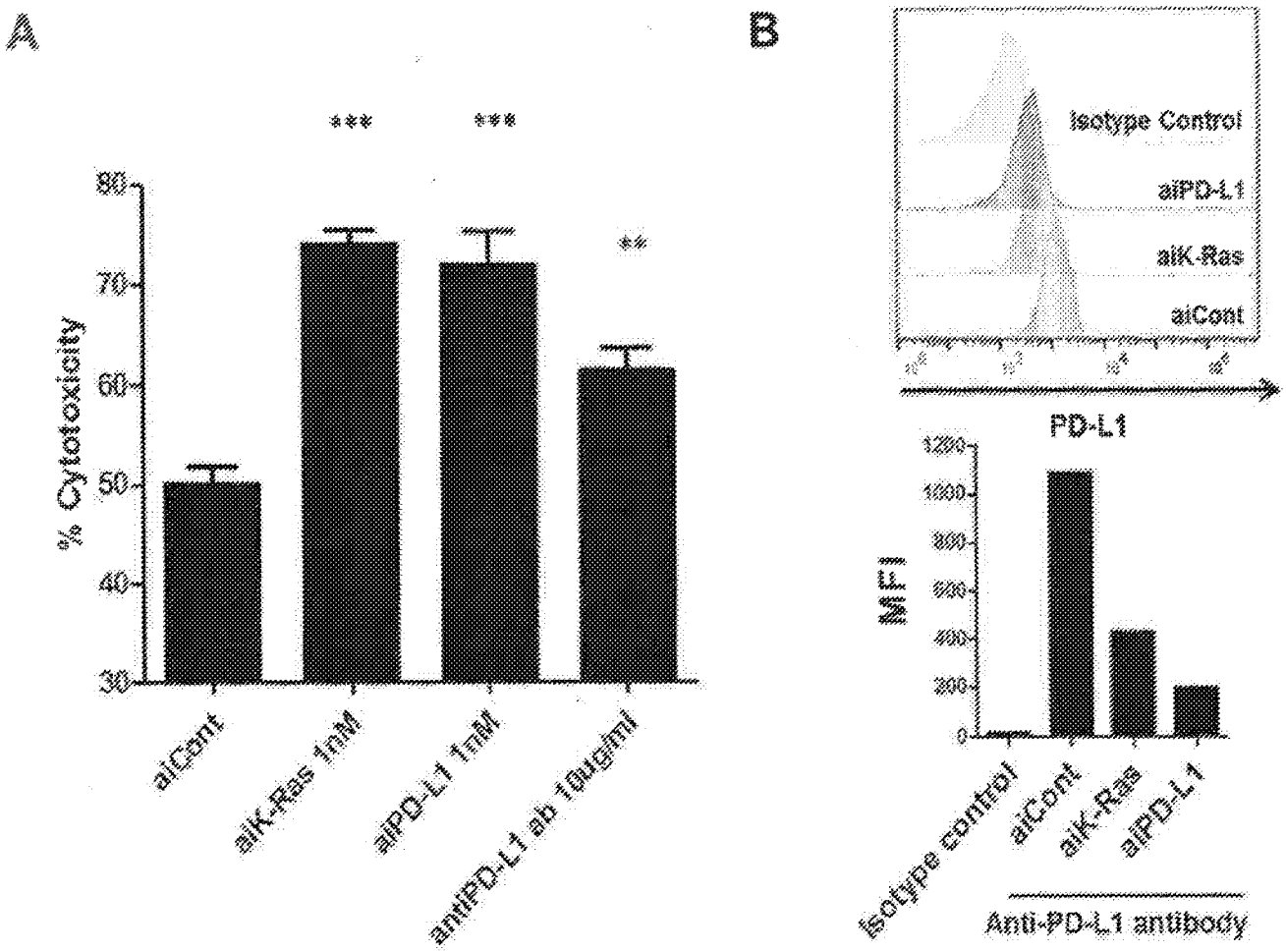

A
B
% Cytotoxicity
80
70
60
50
40
30
***
***
**
aiCont
aiK-Ras 1nM
aiPD-L1 1nM
antiPD-L1 ab 10ug/ml
Isotype Control
aiPD-L1
aiK-Ras
aiCont
PD-L1
MFI
1200
1000
800
600
400
200
0
Isotype control
aiCont
aiK-Ras
aiPD-L1
Anti-PD-L1 antibody

FIG. 7

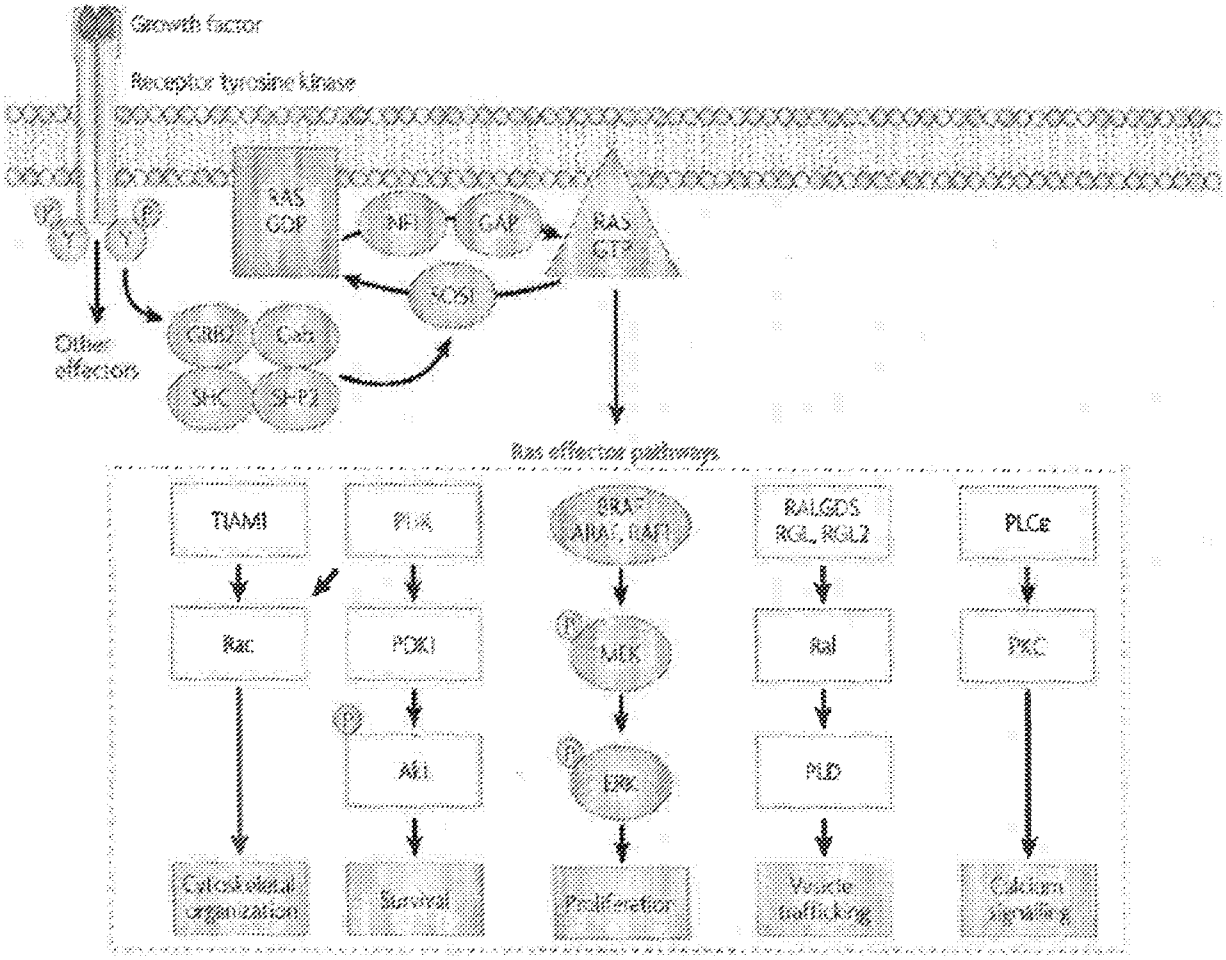

Growth factor
Receptor tyrosine kinase
RAS GDP
NF1
GAP
RAS GTP
SOS1
GRB2
Crk
SHC
SH-P2
Other effectors
Ras effector pathways
TIAM1
PI3K
BRAF ARAF, RAF1
RALGDS RGL, RGL2
PLCε
Rac
PDK1
MEK
Ral
PKC
AKT
ERK
PLD
Cytoskeletal organization
Survival
Proliferation
Vesicle trafficking
Calcium signaling

FIG. 8A

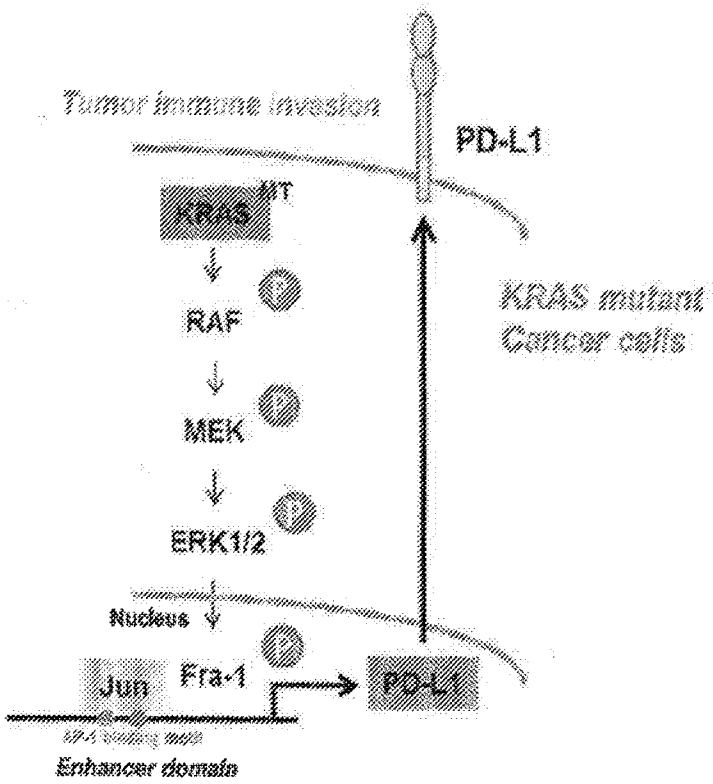

Tumor immune invasion
PD-L1
KRAS MT
RAF
MEK
ERK1/2
KRAS mutant
Cancer cells
Nucleus
Jun
Fra-1
PD-L1
Enhancer domain

FIG. 8B