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(54) Title: TREATMENT OF KRAS-VARIANT CANCERS WITH SEROTONIN UPTAKE INHIBITORS

(57) Abstract: A method of treating a cancer having a KRAS variant in a subject is provided in which an effective amount of one or more serotonin uptake inhibitors is administered to the subject. An example of a serotonin uptake inhibitor is sibutramine. The serotonin uptake inhibitor can be administered alone or in combination with one or more anti-cancer agents. A pharmaceutical composition comprising a serotonin uptake inhibitor and one or more anti-cancer agents is also provided.

WO 2021/236498 A1

TREATMENT OF KRAS-VARIANT CANCERS WITH SEROTONIN UPTAKE INHIBITORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 16/026,411, filed May 18, 2020, the disclosure of which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates to methods and compositions for treating a cancer that has a variant in the KRAS gene.

BACKGROUND

[0003] Ovarian cancer is a cancer that forms in or on an ovary. Ovarian cancer is diagnosed in an estimated 22,240 women in the United States each year. According to the American Cancer Society, ovarian cancer is the fifth leading cause of cancer-related death of women.

[0004] A single base mutation in the 3' untranslated region (3'-UTR) of the KRAS gene has been associated with increased risk of ovarian cancer. The prevalence of this variant is 5.8% among global populations (2). The KRAS variant is an inherited, germline variant that may serve as a genetic marker of increased risk of epithelial ovarian cancer (EOC), with a presence in over 25% of patients with this disease (3,4), although it is controversial as to whether the variant predicts risk (5). The KRAS variant is suggested to be associated with hereditary breast and ovarian cancer (HBOC) (6) in which 61% of HBOC patients harbor the KRAS variant (3), where it may represent a new genetic marker of ovarian risk for patients from HBOC families. Importantly, the KRAS variant impacts responses to various cancer therapies.

[0005] Most importantly, the KRAS variant impacts responses to various cancer therapies. Ovarian tumors with the KRAS variant are resistant to treatment with standard chemotherapies including cisplatin. The standard chemotherapy regimen for treatment of EOC is carboplatin and paclitaxel, based upon prospective randomized clinical trials (7). Tumor microRNA expression patterns have been associated with resistance to platinum-based chemotherapy in patients with EOC (21). Importantly, EOC tumors harboring the KRAS-variant were found to be significantly more resistant to treatment with platinum than those without the KRAS-variant (8).

[0006] There is currently no drug available to specifically treat KRAS-variant ovarian cancer. The present invention provides a solution to this problem.

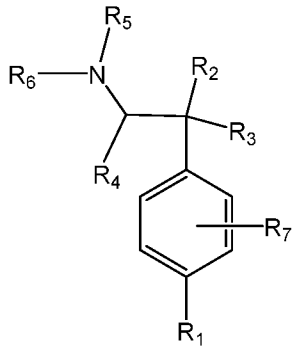
SUMMARY OF THE INVENTION

[0007] In one aspect is provided a method of treating a cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of a serotonin uptake inhibitor, wherein the subject comprises rs61764370 variant of the KRAS gene. In various embodiments, the subject comprises rs61764370 variant of the KRAS gene in cancer cells.

[0008] In some embodiments, the cancer is an ovarian cancer. In a specific embodiment, the ovarian cancer is a hereditary ovarian cancer. In a specific embodiment, the ovarian cancer is an epithelial ovarian cancer.

[0009] In some embodiments, the cancer is a breast cancer.

[0010] In some embodiments, the serotonin uptake inhibitor has a structure according to formula (I):



(I),

wherein R_1 is selected from H, a halide, and a C_1-C_3 perfluorocarbon;

R_2 and R_3 are independently selected from H and a C_1-C_3 hydrocarbon, or R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;

R_4 is selected from H and a C_1-C_6 hydrocarbon optionally substituted with -OH or a halide;

R_5 and R_6 are independently selected from H and a C_1-C_3 hydrocarbon;

R_7 is absent or is selected from a halide and a C_1-C_3 perfluorocarbon. In various embodiments, a pharmaceutically acceptable salt of the compound is provided.

[0011] In a specific embodiment, the serotonin uptake inhibitor has a structure according to formula (I) in which

R_1 is selected from a halide and a C_1-C_3 perfluorocarbon;

R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;

R_4 is a C_1-C_6 hydrocarbon optionally substituted with -OH or a halide;

R_5 and R_6 are independently selected from H and -CH_3;

R_7 is absent or is a halide. In various embodiments, a pharmaceutically acceptable salt of the compound is provided.

[0012] In a specific embodiment, the serotonin uptake inhibitor has a structure according to formula (I) in which

R_1 is a halide;

R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;

R_4 is a C_3-C_6 hydrocarbon optionally substituted with -OH or a halide;

R_5 and R_6 are independently selected from H and -CH_3;

R_7 is absent. In various embodiments, a pharmaceutically acceptable salt of the compound is provided.

[0013] In a specific embodiment, the serotonin uptake inhibitor has a structure according to formula (I) in which

R_1 is -Cl;

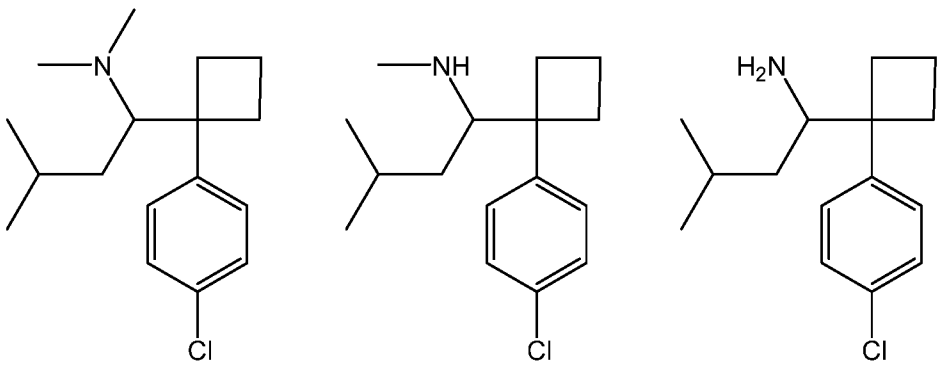
R_2 and R_3 together form a 4-membered carbocycle;

R_4 is a selected from n-propyl, isopropyl, n-butyl, isobutyl, and t-butyl;

R_5 and R_6 are independently selected from H and -CH_3;

R_7 is absent. In various embodiments, a pharmaceutically acceptable salt of the compound is provided.

[0014] In some embodiments, the compound of formula (I) is selected from



sibutramine, desmethylsibutramine, and didesmethylsibutramine.

[0015] In various embodiments, a pharmaceutically acceptable salt of the compound is provided.

[0016] In various embodiments, the serotonin uptake inhibitor is selected from fluoxetine, citalopram, escitalopram, sertraline, norsertraline, fluvoxamine, femoxetine, indalpine, alaproclate, cericlamine, ifoxetine, zimelidine, dapoxetine, etoperidone, desmethylcitalopram, didesmethylcitalopram, and seproxetine. In a specific embodiment, the serotonin uptake inhibitor is sibutramine. In a specific embodiment, the serotonin uptake inhibitor is (R)-sibutramine. In a specific embodiment, the serotonin uptake inhibitor is (S)-sibutramine. In a specific embodiment, the serotonin uptake inhibitor is desmethylsibutramine or didesmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (R)-desmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (S)-desmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (R)-didesmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (S)-didesmethylsibutramine.

[0017] In various embodiments, the serotonin uptake inhibitor is more effective in killing a cancer cell comprising the rs61764370 variant of the KRAS gene than killing a corresponding cancer cell that does not comprise the rs61764370 variant of the KRAS gene.

[0018] In various embodiments, the serotonin uptake inhibitor is more effective in inhibiting survival of a cancer cell comprising the rs61764370 variant of the KRAS gene than in inhibiting survival of a corresponding cancer cell that does not comprise the rs61764370 variant of the KRAS gene. In a specific embodiment, the administration of the serotonin uptake inhibitor results in inhibition of the growth of a tumor of the cancer.

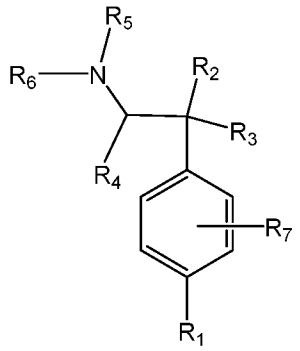
[0019] In some embodiments, the method further comprises administering one or more additional anti-cancer agents. In some embodiments, the serotonin uptake inhibitor and the additional anti-cancer agent are administered concurrently. In some embodiments, the serotonin uptake inhibitor and the additional anti-cancer agent are administered in one composition. In some embodiments, the serotonin uptake inhibitor and the additional anti-cancer agent are administered sequentially.

[0020] In various embodiments, the additional anti-cancer agent is a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is selected from paclitaxel, a PARP inhibitor, a platinum agent, and doxorubicin. In some embodiments, the method further comprises administering to the subject one or more additional anti-cancer treatments selected from a radiation therapy, an immunotherapy and a gene therapy. In some embodiments, the serotonin uptake inhibitor is administered orally.

[0021] In various embodiments, the subject is a human.

[0022] In another aspect is provided a composition comprising a serotonin uptake inhibitor and one or more additional anti-cancer agents.

[0023] In some embodiments, the serotonin uptake inhibitor has a structure according to formula (I):



(I),

wherein R_1 is selected from H, a halide, and a C_1-C_3 perfluorocarbon;

R_2 and R_3 are independently selected from H and a C_1-C_3 hydrocarbon, or R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;

R_4 is selected from H and a C_1-C_6 hydrocarbon optionally substituted with -OH or a halide;

R_5 and R_6 are independently selected from H and a C_1-C_3 hydrocarbon;

R_7 is absent or is selected from a halide and a C_1-C_3 perfluorocarbon. In various embodiments, a pharmaceutically acceptable salt of the compound is provided.

[0024] In a specific embodiment, the serotonin uptake inhibitor of the pharmaceutical composition has a structure according to formula (I) in which:

R_1 is selected from a halide and a C_1-C_3 perfluorocarbon;

R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;

R_4 is a C_1-C_6 hydrocarbon optionally substituted with -OH or a halide;

R_5 and R_6 are independently selected from H and -CH_3;

R_7 is absent or is a halide. In various embodiments, a pharmaceutically acceptable salt of the compound is provided.

[0025] In a specific embodiment, the serotonin uptake inhibitor of the pharmaceutical composition has a structure according to formula (I) in which:

R_1 is a halide;

R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;

R_4 is a C_3-C_6 hydrocarbon optionally substituted with -OH or a halide;

R_5 and R_6 are independently selected from H and -CH_3;

R_7 is absent. In various embodiments, a pharmaceutically acceptable salt of the compound is provided.

[0026] In a specific embodiment, the serotonin uptake inhibitor of the pharmaceutical composition has a structure according to formula (I) in which:

R_1 is -Cl;

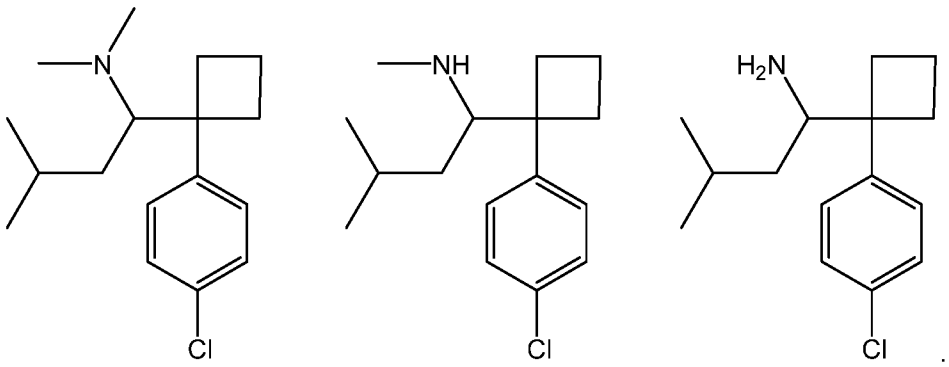
R_2 and R_3 together form a 4-membered carbocycle;

R_4 is a selected from n-propyl, isopropyl, n-butyl, isobutyl, and t-butyl;

R_5 and R_6 are independently selected from H and $-CH_3$;

R_7 is absent. In various embodiments, a pharmaceutically acceptable salt of the compound is provided.

[0027] In a specific embodiment, the compound of formula (I) is selected from



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[0028] In various embodiments, a pharmaceutically acceptable salt of the compound is provided. In some embodiments, the serotonin uptake inhibitor is selected from fluoxetine, citalopram, escitalopram, sertraline, norsertraline, fluvoxamine, femoxetine, indalpine, alaproclate, cericlamine, ifoxetine, zimelidine, dapoxetine, etoperidone, desmethylcitalopram, didesmethylcitalopram, and seproxetine. In a specific embodiment, the serotonin uptake inhibitor is sibutramine. In a specific embodiment, the serotonin uptake inhibitor is (R)-sibutramine. In a specific embodiment, the serotonin uptake inhibitor is (S)-sibutramine. In a specific embodiment, the serotonin uptake inhibitor is desmethylsibutramine or didesmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (R)-desmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (S)-desmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (R)-didesmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (S)-didesmethylsibutramine. In a specific embodiment, the additional anti-cancer agent is paclitaxel, a PARP inhibitor, a platinum agent, or doxorubicin.

[0029] In various embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier or excipient.

BRIEF DESCRIPTION OF DRAWINGS

[0030] FIG. 1 is a diagram of the KRAS variant. The KRAS-variant is a miRNA-binding site mutation in the 3'-UTR of the KRAS gene. The mutation interferes with binding of let-7 family of miRNAs. If let-7 doesn't bind to the 3'UTR it is unable to inhibit KRAS expression.

[0031] FIG. 2 is a graph showing the effects of sibutramine in a clonogenic survival assay. KRAS-variant ovarian cancer cells are more sensitive to sibutramine than non-KRAS variant isogenic control ovarian cancer cells (SKOV isogenic control).

[0032] FIGs. 3A and 3B are graphs showing the effects of sibutramine in a tumor xenograft study. FIG. 3A shows tumor growth in response to sibutramine treatment in the athymic nude mice implanted with SKOV3 xenograft with wild type KRAS. FIG. 3B shows tumor growth in response to sibutramine treatment in the athymic nude mice implanted with SKOV3 xenograft harboring the KRAS-variant. Error bars were not included given low numbers of animals (n=2-3 per treatment group). These figures demonstrate that KRAS variant xenografts are sensitive to sibutramine.

[0033] FIGs. 4A and 4B are images of the crystal structures of serotonin transporter (SERT) and 5'-3' Exoribonuclease 2 (XRN2), respectively.

[0034] FIGs. 5A-5C are schematics of the chemical structures of sibutramine (FIG. 5A) and its two metabolites, desmethylsibutramine (FIG. 5B) and didesmethylsibutramine (FIG. 5C).

[0035] FIGs. 6A-6B are graphs showing the inhibitory effects of fluoxetine (FE), sibutramine (SE), cisplatin (CisP) (FIG. 6A), and paclitaxel (P) (FIG. 6B) on the proliferation of MCF-7 cells, with cisplatin (CisP) and paclitaxel (P) used as the positive control.

[0036] FIGs. 7A-7B are graphs showing the inhibitory effects of fluoxetine (FE), sibutramine (SE), cisplatin (CisP) (FIG. 7A), and paclitaxel (P) (FIG. 7B) on the proliferation of ZR-75-1 cells, with cisplatin (CisP) and paclitaxel (P) used as the positive control.

DETAILED DESCRIPTION

[0037] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0038] The terms "a," "an," and "the" do not denote a limitation of quantity, but rather denote the presence of "at least one" of the referenced item.

[0039] The terms "patient", "individual", "subject", "mammal", and "animal" are used interchangeably herein and refer to mammals, including, without limitation, human, veterinary

animals (e.g., cats, dogs, cows, horses, sheep, pigs, etc.) and experimental animal models (e.g., mouse, rabbit, rat). In a preferred embodiment, the subject is a human.

[0040] The terms "treat" or "treatment" of a state, disorder or condition include: (1) preventing, delaying, or reducing the incidence and/or likelihood of the appearance of at least one clinical or sub-clinical symptom of the state, disorder or condition developing in a subject that may be afflicted with or predisposed to the state, disorder or condition but does not yet experience or display clinical or subclinical symptoms of the state, disorder or condition; or (2) inhibiting the state, disorder or condition, i.e., arresting, reducing or delaying the development of the disease or a relapse thereof (in case of maintenance treatment) or at least one clinical or sub-clinical symptom thereof; or (3) relieving the disease, i.e., causing regression of the state, disorder or condition or at least one of its clinical or sub-clinical symptoms. The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician.

[0041] The term "effective amount" as used herein, refers to an amount of a serotonin uptake inhibitor that is effective to treat a cancer in a subject.

[0042] The term "pharmaceutically acceptable", as used herein, refers to molecular entities and other ingredients of such compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a mammal (e.g., a human). Preferably, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans.

[0043] The term "carrier" or "a pharmaceutically acceptable carrier" as used herein, refers to any clinically useful solvents, diluents, adjuvants, excipients, recipients, vehicles and the like for use in preparing admixtures of a pharmaceutical composition.

[0044] In one aspect the invention provides a method of treating a cancer in a subject, in which a cell of the cancer comprises a variant in the KRAS gene. An effective amount of one or more serotonin uptake inhibitors is administered to the subject.

[0045] In another aspect, the invention provides a method of treating a cancer in a subject in need thereof. An effective amount of a serotonin uptake inhibitor is administered to the subject. The subject has the rs61764370 variant of the KRAS gene.

[0046] As used herein, the terms "serotonin uptake inhibitor" refer to any compound that is capable of directly or indirectly inhibiting the reupdate of serotonin. A serotonin uptake inhibitor may inhibit the uptake of serotonin by blocking the action of the serotonin transporter. Serotonin, or 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter. In some embodiments, the serotonin uptake inhibitor also inhibits the uptake of other neurotransmitter(s) such as dopamine and norepinephrine. In some embodiments, the serotonin uptake inhibitor is a selective serotonin reuptake inhibitor or SSRI.

[0047] Serotonin transporter (SERT), also known as the sodium-dependent serotonin transporter and solute carrier family 6 member 4, is a protein encoded by the SLC6A4 gene in human. SERT is a type of monoamine transporter protein that transports serotonin from the synaptic cleft to the presynaptic neuron.

[0048] The KRAS gene encodes a KRAS protein which is a p21 GTPase belonging to the small GTPase superfamily. The KRAS protein functions as an on/off switch relaying the extracellular signals of receptor tyrosine kinases (i.e. EGFR) and initiating the cascade of signal transduction. Activated KRAS phosphorylates and activates Raf, which in turn phosphorylates and activates MEK, which eventually promotes cellular survival. The KRAS gene belongs to a class of genes known as oncogenes, which when mutated can cause normal cells to become cancerous. A single amino acid substitution, or a single nucleotide substitution, may be responsible for an activating mutation. Abnormally active KRAS protein may direct cells to proliferate in an uncontrolled way. Mutations in the KRAS gene have been associated with various types of cancer.

[0049] In various embodiments, the cancer is ovarian cancer. In various embodiments, the cancer is breast cancer. In various embodiments, the cancer is a glioma cancer. In various embodiments, the cancer is a prostate cancer. In various embodiments, the cancer is a lung cancer. In various embodiments, the cancer is a colorectal cancer. In various embodiments, the cancer is a head and neck cancer.

[0050] In a specific embodiment, the cancer is an ovarian cancer. The ovarian cancer can be an epithelial ovarian cancer (EOC). Epithelial ovarian cancer starts on the surface of the ovaries, and may account for at least 80% of ovarian cancer cases. The EOC can be a high-grade serous carcinoma (HGSC), an endometrioid carcinoma (EC), a clear cell carcinoma (CCC), a mucinous carcinoma (MC) or a low-grade serous (LGSC). The ovarian cancer can be a germ cell ovarian cancer that forms in the germ (egg) cells of the ovary. The ovarian cancer can be a stromal ovarian cancer that arises from the connective tissue cells that hold the ovary together and produce hormones. The ovarian cancer can be a mixed ovarian cancer. Further, the ovarian cancer can be a hereditary breast and ovarian cancer (HBOC). HBOC is a condition where individuals develop breast and/or ovarian cancers due to inherited genetic abnormalities. HBOC may primarily be associated with mutations in the *BRCA1* or *BRCA2* genes.

[0051] In various embodiments, the variant in the KRAS gene is a mutation in the miRNA-binding site in the 3' untranslated region (3' UTR) of the KRAS gene. MicroRNAs (miRNAs) are a class of short noncoding RNAs (about 22 nucleotides) that are aberrantly expressed in virtually all cancer types, where they can function as a novel class of oncogenes or tumor suppressor genes (18-20). The binding of miRNAs to their target mRNAs is critical for the regulation of mRNA levels and subsequent protein expression, and this regulation can

be affected by mutations such as single-nucleotide polymorphisms (SNPs) in miRNA binding sites in the 3′UTR of genes. The regulatory mechanisms of miRNAs are complex. Although not wishing to be bound by theory, it is known that an inability of miRNAs to bind to their seed sequences leads to their downregulation via degradation.

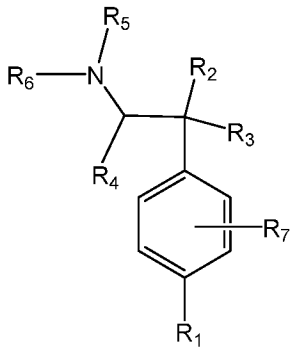
[0052] In various embodiments, the miRNA binding site is specific for a miRNA from the let-7 miRNA family. The let-7 miRNA may be a let-7a, let-7b, let-7d or let-7g miRNA.

[0053] The let-7 family of miRNAs are global genetic regulators important in regulating cell proliferation, cell cycle, apoptosis, metabolism, and stemness. The let-7 family of miRNAs have been shown to regulate KRAS. Members of the let-7 family are known to be susceptible to degradation via the XRN-1 (5'-3' exoribonuclease 1) nuclease, both in human cells and in *C. elegans*. In addition, tumors harboring the KRAS variant were found to have lower levels of let-7a, b, d, and g. A mutation in the miRNA binding site in the KRAS gene may lead to defective binding of the let-7 family of miRNAs, resulting in upregulation of the KRAS gene, which may drive carcinogenesis. The presence of the KRAS variant may also lead to downregulation of the let-7 family of miRNAs, likely via a degradative mechanism (1).

[0054] The presence of such KRAS variant may impact responses of cancers to various cancer therapies. For example, tumors in epithelial ovarian cancer harboring the KRAS variant were found to be significantly more resistant to treatment with platinum than those without the KRAS variant (8).

[0055] In a specific embodiment, the variant in the KRAS gene is rs61764370. rs61764370 refers to a single-nucleotide polymorphism (SNP) of T>G at position chr12:25207290 (GRCh38 38.1/141) which is in the 3'UTR miRNA binding site of the KRAS gene. As a non-limiting example, the KRAS variant may be a miRNA-binding site mutation in the 3'-UTR of the KRAS gene as shown in FIG. 1.

[0056] In some embodiments, the serotonin uptake inhibitor has a structure according to formula (I):



(I),

where R_1 is selected from H, a halide, and a C_1-C_3 perfluorocarbon;
R_2 and R_3 are independently selected from H and a C_1-C_3 hydrocarbon, or R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;
R_4 is selected from H and a C_1-C_6 hydrocarbon optionally substituted with -OH or a halide;
R_5 and R_6 are independently selected from H and a C_1-C_3 hydrocarbon;
R_7 is absent or is selected from a halide and a C_1-C_3 perfluorocarbon,
or a pharmaceutically acceptable salt thereof.

[0057] In some embodiments, the serotonin uptake inhibitor is sibutramine, fluoxetine, citalopram, escitalopram, sertraline, norsertraline, fluvoxamine, femoxetine, indalpine, alaproclate, cericlamine, ifoxetine, zimelidine, dapoxetine, etoperidone, desmethylcitalopram, didesmethylcitalopram, or seproxetine, or an analog, derivative or metabolite thereof.

[0058] In a specific embodiment, the serotonin uptake inhibitor is sibutramine. Sibutramine is a racemic mixture of R,S stereoisomers of the phenethylamine class of drugs and is subject to rapid first-pass metabolism in humans. Sibutramine was developed to treat obesity and marketed under the name Meridia (for example see (16)). There are two major metabolites of this drug, both of which are also optically active: desmethylsibutramine and didesmethylsibutramine. Sibutramine and its metabolites are known to inhibit uptake of dopamine, norepinephrine, and serotonin (16). The chemical structures of sibutramine, desmethylsibutramine and didesmethylsibutramine are shown in FIGs. 5A-5C, respectively. The present disclosure encompasses sibutramine, sibutramine metabolites, and their enantiomers, as well as analogs, derivatives, pharmaceutically acceptable salts, solvates, hydrates, clathrates, or prodrugs thereof.

[0059] Sibutramine was withdrawn for use as an anti-obesity drug due to a risk benefits analysis showing that subjects taking sibutramine lost only 5-10 pounds during the 3-4 years they were enrolled on the SCOUT trial. The inventors of the present disclosure, however, have identified a surprising and unexpected activity of sibutramine in cancer treatment.

[0060] In a specific embodiment, the serotonin uptake inhibitor administered is (R)-sibutramine. In another specific embodiment, the serotonin uptake inhibitor administered is (S)-sibutramine.

[0061] In some embodiments, the serotonin uptake inhibitor administered is desmethylsibutramine or didesmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor administered is (R)-desmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor administered is (S)-desmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (R)-didesmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (S)-didesmethylsibutramine.

[0062] In some embodiments, the serotonin uptake inhibitor is an analog of sibutramine that is brain-penetrable. Brain-penetrable compounds are capable of crossing the blood-brain barrier. Brain-penetrable analogs of sibutramine may be particularly useful in treating brain cancers (e.g., glioma) that harbor the KRAS variant.

[0063] In various embodiments, the serotonin uptake inhibitor is more effective in killing the cell of the cancer with the KRAS variant, e.g., rs61764370, than in killing a corresponding cell of a cancer that does not comprise the same variant in the KRAS gene.

[0064] In various embodiments, the serotonin uptake inhibitor inhibits the growth of a tumor of the cancer.

[0065] Although not wishing to be bound by theory, a serotonin uptake inhibitor (e.g., sibutramine) may exert anti-cancer activity through synthetic lethality with the KRAS mutation. Synthetic lethality is based upon the premise that genetic mutations in cancers synergize with specific drugs to result in tumor kill. For example, BRCA1 and 2 mutations exhibit synthetic lethality with poly-ADP-ribose polymerase 1 (PARP1) inhibitors, resulting in FDA approval of the PARP inhibitor Olaparib for the treatment of BRCA1/2 mutant ovarian and breast cancer. It is hypothesized that sibutramine may act to inhibit a target protein of the KRAS-MAPK pathway in a direct or indirect manner, thereby selectively inducing lethality of KRAS variant cancers.

[0066] A serotonin uptake inhibitor (e.g., sibutramine) may target one or more targets in the serotonin transporter (SERT) pathway or the miRNA processing pathway (e.g., enzymes or complex). Its target location(s) may be extracellular and/or intracellular. It may target the central nervous system (CNS) and/or other tissue types.

[0067] In various embodiments, administering a serotonin uptake inhibitor to the subject for cancer treatment does not lead to weight loss.

[0068] In various embodiments, administering a serotonin uptake inhibitor to the subject for cancer treatment does not lead to one or more of the following side effects: flu symptoms, runny or stuffy nose, sore throat, cough, fast or pounding heartbeats, high blood pressure, new or worsening shortness of breath, seizure (convulsions), fast or uneven heartbeats, dry mouth, upset stomach, loss of appetite, constipation, stomach pain, headache, back pain, joint pain, nervousness, dizziness, depression, trouble sleeping (insomnia), skin rash, and warmth, redness, or tingly feeling under the skin.

Combination therapy

[0069] In various embodiments, the method described herein further includes administering one or more anti-cancer agents. Combination therapy of serotonin uptake

inhibitors with other anti-cancer agents may exert synergistic or additive effects against the cancer.

[0070] In some embodiments, the serotonin uptake inhibitor and the anti-cancer agent may be administered concurrently. In other embodiments, the serotonin uptake inhibitor and the anti-cancer agent may be administered sequentially, for example the serotonin uptake inhibitor is administered before the anti-cancer agent or the serotonin uptake inhibitor is administered after the anti-cancer agent.

[0071] In some embodiments, the anti-cancer agent is a chemotherapeutic agent. As used herein, the term "chemotherapeutic agent" refers to any agent that has therapeutic usefulness in the treatment of cancer. Chemotherapeutic agents as used herein encompass both chemical and biological agents. Such agents may function to inhibit a cellular activity upon which the cancer cell depends for continued survival.

[0072] Examples of chemotherapeutic agents include alkylating agents such as thiotepa, cyclophosphamide, and temozolomide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodepa, carboquone, meturedepa, and uredepa; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins (e.g., mitomycin C), mycophenolic acid, Nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites such as methotrexate and 5-FU; folic acid analogs such as denopterin, aminopterin, methotrexate, pteropterin, and trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine (cytosine arabinoside or ara-C), capecitabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, gemcitabine; folic acid replenisher such as folinic acid (leucovorin); nitrogen mustards such as chlorambucil, chlornaphazine, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, mannomustine and uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; members of taxoid or taxane family, such as paclitaxel (TAXOL®), docetaxel (TAXOTERE®) and analogs thereof; platinum agents such as cisplatin and carboplatin; vinca alkaloids such as vindesine, vinblastine, vincristine, and vinorelbine; topoisomerase inhibitors such as doxorubicin, daunorubicin (daunomycin), dactinomycin, teniposide, epirubicin, etoposide (VP-16), idarubicin, irinotecan, mitoxantrone, topotecan, razoxane, sobuzoxane and 9-nitrocamptothecin;androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, and testolactone; anti-

adrenals such as aminoglutethimide, mitotane, and trilostane; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; dacarbazine; demecolcine; diaziquone; difluoromethylornithine (DMFO); edatrexate; eflornithine; elliptinium acetate; etoglucid; 2-ethylhydrazide; gacytosine; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitobronitol; mitolactol; mitoguazone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; procarbazine; polysaccharide-K (PSK); sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; urethan; pipobroman; ibandronate; retinoic acid; and inhibitors of receptor tyrosine kinases and/or angiogenesis, including sorafenib (NEXAVAR®), Sunitinib (SUTENT®), pazopanib (VOTRIENT™), toceranib (PALLADIA™), vandetanib (ZACTIMA™, cediranib (RECENT3N®), regorafenib (BAY 73-4506), axitinib (AG013736), lestaurtinib (CEP-701), erlotinib (TARCEVA®), gefitinib (IRESSA™), Afatinib (TOVOK™), lapatinib (TYKERB®), neratinib (HKI-272); PARP inhibitors such as olaparib, and the like, and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0073] In a specific embodiment, the chemotherapeutic agent is paclitaxel, a PARP inhibitor, a platinum agent, or doxorubicin.

[0074] In a specific embodiment, the chemotherapeutic agent used in the combination therapy is paclitaxel.

[0075] In some embodiments, the chemotherapeutic agent used in the combination therapy is a PARP inhibitor or a platinum agent.

[0076] PARP inhibitors are a group of inhibitors of the enzyme poly (ADP-ribose) polymerase (PARP). Exemplary PARP inhibitors that can be used in the methods described herein include olaparib, rucaparib, niraparib, talazoparib, veliparib, pamiparib (BGB-290), Iniparib (BSI 201), CEP 9722, E7449, AG-14361, INO-1001, A-966492, PJ34 HCl, UPF 1069, AZD2461, ME0328, BGP-15 2HCl, Niraparib tosylate, NU1025, NVP-TNKS 656, NMS-P118, benzamide and picolinamide, and pharmaceutically acceptable salts, acids or derivatives thereof. In a specific embodiment, the PARP inhibitor is olaparib.

[0077] Platinum agents that can be used in the combination therapy described herein include, but are not limited to carboplatin, cis-platin, oxaliplatin, lobaplatin, enloplatin and miboplatin, and pharmaceutically acceptable salts, acids or derivatives thereof. In a specific embodiment, the platinum agent is carboplatin. In another specific embodiment, the platinum agent is cis-platin.

[0078] Additional chemotherapeutic agents that may be used in the combination therapy described herein include, but are not limited to, docetaxel, albumin bound paclitaxel, altretamine, bleomycin, capecitabine, cyclophosphamide, dactinomycin, etoposide, gemcitabine, ifosfamide, irinotecan, liposomal doxorubicin, melphalan, pemetrexed,

topotecan, vinblastine, vincristine and vinorelbine, and the like, and pharmaceutically acceptable salts, acids or derivatives thereof.

[0079] In some embodiments, the anti-cancer agent is an anti-hormonal agent. The anti-hormonal agent may be anti-estrogen or selective estrogen receptor modulator such as tamoxifen, raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene; an aromatase inhibitor such as letrozole, anastrozole, and exemestane; a luteinizing-hormone-releasing hormone (LHRH) agonist such as goserelin, leuprolide and triptorelin; or an anti-androgen such as flutamide, nilutamide, bicalutamide, leuprohde, and goserelin; or a pharmaceutically acceptable salt, acid or derivative thereof.

[0080] In some embodiments, the anti-cancer agent is a therapeutic antibody. The therapeutic antibody may be 3F8, abagovomab, adecatumumab, afutuzumab, alemtuzumab, altumomab, amatuximab, anatumomab, arcitumomab, bavituximab, bectumomab, bevacizumab, bivatuzumab, blinatumomab, brentuximab, cantuzumab, catumaxomab, CC49, cetuximab, citatuzumab, cixutumumab, clivatuzumab, conatumumab, dacetuzumab, dalotuzumab, daratumumab, detumomab, drozitumab, duligotumab, dusigitumab, ecromeximab, elotuzumab, ensituximab, ertumaxomab, etaracizumab, farletuzumab, ficlatuzumab, figitumumab, flanvotumab, futuximab, ganitumab, gemtuzumab, girentuximab, glembatumumab, ibritumomab, igovomab, imgatuzumab, indatuximab, inotuzumab, intetumumab, ipilimumab (YERVOY®, MDX-010, BMS-734016 and MDX-101), iratumumab, labetuzumab, lexatumumab, lintuzumab, lorvotuzumab, lucatumumab, mapatumumab, matuzumab, milatuzumab, minretumomab, mitumomab, moxetumomab, naptumomab, narnatumab, necitumumab, nimotuzumab, nofetumomab, obinutuzumab, ocaratuzumab, ofatumumab, olaratumab, onartuzumab, oportuzumab, oregovomab, panitumumab, parsatuzumab, patritumab, pemtumomab, pertuzumab, pintumomab, pritumumab, racotumomab, radretumab, rilotumumab, rituximab, robatumumab, satumomab, sibrotuzumab, siltuximab, simtuzumab, solitomab, tacatuzumab, taplitumomab, tenatumomab, teprotumumab, tigatuzumab, tositumomab, trastuzumab, tucotuzumab, ublituximab, veltuzumab, vorsetuzumab, votumumab, or zalutumumab, or a variant or a fragment thereof.

[0081] In some embodiments, the anti-cancer agent is an angiogenesis inhibitor. The angiogenesis inhibitor may be axitinib, bevacizumab, cabozantinib, everolimus, lenalidomide, lenvatinib mesylate, pazopanib, ramucirumab, regorafenib, sorafenib, sunitinib, thalidomide, vandetanib, or ziv-aflibercept, or a pharmaceutically acceptable salt, acid or derivative thereof.

[0082] In some embodiments, the methods described herein also includes treating the subject with one or more of radiation therapy, immunotherapy, and gene therapy.

[0083] Radiation therapy that can be used in the methods described herein may include external beam radiation, intensity modulated radiation therapy (IMRT) and any form of

radiosurgery including Gamma Knife, Cyberknife, Linac, and interstitial radiation (e.g. implanted radioactive seeds, GliaSite balloon).

[0084] Cancer immunotherapy improves the immune system's ability to fight cancer. In some embodiments, immunotherapy involves the use of an immunotherapeutic agent which is an immune checkpoint inhibitor. Exemplary immune checkpoint inhibitors that may be used in the methods described herein include but are not limited to, anti-PD-1 agents, anti-PD-L1 agents, or anti-CTLA-4 agents. Programmed Death-1 (PD-1) is an immunoinhibitory receptor expressed predominantly on previously activated T cells *in vivo*, Programmed Death Ligand-1 (PD-L1) is one of two cell surface glycoprotein ligands for PD-1 (the other being PD-L2) that downregulate T cell activation and cytokine secretion upon binding to PD-1. Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) is an immunoinhibitory receptor expressed exclusively on T cells *in vivo*, and binds to two ligands, CD80 and CD86 (also called B7-1 and B7-2, respectively). In specific embodiments, the immune checkpoint inhibitors may be an anti-PD-1 antibody such as pembrolizumab, nivolumab, and cemiplimab; an anti-PD-L1 antibody such as atezolizumab, avelumab and durvalumab, or an anti-CTLA-4 antibody such as ipilimumab.

[0085] As used herein, the term "gene therapy" refers to the administration of nucleic acid (either native or modified) into a patient's cells to treat cancer. Gene therapies may be used to supply a functional copy of a mutated gene, inactivate a gene by targeting the gene's DNA directly or targeting the mRNA transcript from the gene, or introduce a new gene into a target cell. Exemplary gene therapies that may be used in the methods described herein include (1) tumor suppressor gene therapies that restore cell control through replacing tumor suppressor genes (e.g., *p53* and *WWOX*); (2) oncogene inhibition therapies that inactivate dominant oncogenes (e.g., *EGFR* and *CLDN3*); (3) suicide gene therapies by an enzyme/prodrug system (e.g., herpes simplex virus-thymidine kinase (HVS-TK) system) or activating expression of a toxin (e.g., diphtheria toxin-A); (4) antiangiogenic gene therapy by delivering VEGFRs or angiogenesis inhibitors (e.g., angiostatin and endostatin); (5) immunopotentiation gene therapies by strengthening the immune response to tumor cells (e.g., chimeric antigen receptor T cell therapy), augmenting the expression of tumor antigens or the production of cytokines, interleukins (e.g., IL-21) and growth factors; (6) multi-drug resistance (MDR) associated gene therapies to knockdown genes such as *MDR1* and *survivin*; and (7) oncolytic virotherapies that utilize oncolytic viruses to preferentially kill tumor cells (e.g., vesicular stomatitis virus).

Administration and Dosing

[0086] Any suitable route of administration may be employed for providing the subject with an effective dosage of the serotonin uptake inhibitor. For example, the serotonin uptake

inhibitor may be administered by an oral, mucosal (e.g., nasal, sublingual, buccal, rectal, and vaginal), parenteral (e.g., intravenous, intramuscular, subcutaneous), or transdermal route, or a combination thereof.

[0087] In some embodiments, the serotonin uptake inhibitor is administered orally.

[0088] In some embodiments, the serotonin uptake inhibitor is administered to the subject in an amount from about 0.1 mg to about 60 mg. As a non-limiting example, the serotonin uptake inhibitor may be administered to the subject in an amount from about 0.1 mg to about 20 mg, from about 0.5 mg to about 30 mg, from about 1 mg to about 20 mg, from about 1 mg to about 30 mg, from about 1 mg to about 40 mg, from about 2 mg to about 15 mg, from about 2 mg to about 30 mg, from about 2 mg to about 45 mg, from about 5 mg to about 20 mg, from about 5 mg to about 40 mg, from about 8 mg to about 30 mg, from about 8 mg to about 45 mg, from about 10 mg to about 30 mg, from about 10 mg to about 50 mg, from about 15 mg to about 30 mg, from about 15 mg to about 45 mg, from about 20 mg to about 40 mg, from about 20 mg to about 60 mg, from about 25 mg to about 50 mg, or from about 30 mg to about 60 mg.

[0089] In some embodiments, the serotonin uptake inhibitor is administered to the subject in any one of the above-described amounts every 8 hours, every 12 hours, every 24 hours, every 36 hours, every 48 hours, or every 60 hours. In some embodiments, the serotonin uptake inhibitor is administered to the subject in any one of the above-described amounts once daily, twice daily or three times daily.

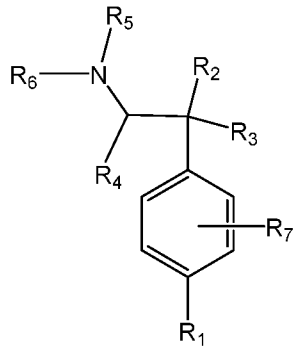
[0090] In one specific embodiment, the serotonin uptake inhibitor is administered to the subject in subject in any one of the above-described amounts once daily.

[0091] In various embodiments, the subject being treated is a human. The subject may be an adult. The subject may be a child. Subjects with KRAS variant may be pre-selected by genetic testing specific for identifying the presence of the KRAS variant.

Pharmaceutical Compositions

[0092] In one aspect the invention provides a pharmaceutical composition comprising a serotonin uptake inhibitor and one or more anti-cancer agents.

[0093] In some embodiments, the serotonin uptake inhibitor in the pharmaceutical composition has a structure according to formula (I):



(I)

R_1 is selected from H, a halide, and a C_1-C_3 perfluorocarbon. R_2 and R_3 are independently selected from H and a C_1-C_3 hydrocarbon, or R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon. R_4 is selected from H and a C_1-C_6 hydrocarbon optionally substituted with -OH or a halide. R_5 and R_6 are independently selected from H and a C_1-C_3 hydrocarbon. R_7 is absent or is selected from a halide and a C_1-C_3 perfluorocarbon. A pharmaceutically acceptable salt of the serotonin uptake inhibitor may be used.

[0094] R_1 may be selected from a halide and a C_1-C_3 perfluorocarbon. R_2 and R_3 may together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon. R_4 may be a C_1-C_6 hydrocarbon optionally substituted with -OH or a halide. R_5 and R_6 may be independently selected from H and $-CH_3$; R_7 may be absent, or may be a halide. A pharmaceutically acceptable salt of the serotonin uptake inhibitor may be used.

[0095] Alternatively, R_1 may be a halide. R_2 and R_3 together may form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon. R_4 may be a C_3-C_6 hydrocarbon optionally substituted with -OH or a halide. R_5 and R_6 may independently be selected from H and $-CH_3$. R_7 may be absent. A pharmaceutically acceptable salt of the serotonin uptake inhibitor may be used.

[0096] Alternatively, R_1 may be -Cl. R_2 and R_3 together may form a 4-membered carbocycle. R_4 may be n-propyl, isopropyl, n-butyl, isobutyl, or t-butyl. R_5 and R_6 may be H or $-CH_3$. R_7 may be absent. A pharmaceutically acceptable salt of the serotonin uptake inhibitor may be used.

[0097] The compound of formula (I) may be sibutramine, desmethylsibutramine, or didesmethylsibutramine.

[0098] In some embodiments, the serotonin uptake inhibitor in the pharmaceutical composition is sibutramine, fluoxetine, citalopram, escitalopram, sertraline, norsertraline,

fluvoxamine, femoxetine, indalpine, alaproclate, cericlamine, ifoxetine, zimelidine, dapoxetine, etoperidone, desmethylcitalopram, didesmethylcitalopram, or seproxetine, or an analog, derivative or metabolite thereof.

[0099] In some embodiments, the serotonin uptake inhibitor in the pharmaceutical composition is sibutramine. In a specific embodiment, the serotonin uptake inhibitor is (R)-sibutramine. In another specific embodiment, the serotonin uptake inhibitor is (S)-sibutramine.

[00100] In some embodiments, the serotonin uptake inhibitor in the pharmaceutical composition is desmethylsibutramine or didesmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (R)-desmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (S)-desmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is (R)- didesmethylsibutramine. In a specific embodiment, the serotonin uptake inhibitor is the serotonin uptake inhibitor is (S)-didesmethylsibutramine.

[00101] In some embodiments, the anti-cancer agent in the pharmaceutical composition is a chemotherapeutic agent. Examples of chemotherapeutic agents include alkylating agents such as thiotepa, cyclophosphamide, and temozolomide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodepa, carboquone, meturedepa, and uredepa; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins (e.g., mitomycin C), mycophenolic acid, Nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites such as methotrexate and 5-FU; folic acid analogs such as denopterin, aminopterin, methotrexate, pteropterin, and trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine (cytosine arabinoside or ara-C), capecitabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, gemcitabine; folic acid replenisher such as folinic acid (leucovorin); nitrogen mustards such as chlorambucil, chlornaphazine, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, mannomustine and uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; members of taxoid or taxane family, such as paclitaxel (TAXOL®), docetaxel (TAXOTERE®) and analogs thereof; platinum agents such as cisplatin and carboplatin; vinca alkaloids such as vindesine, vinblastine, vincristine, and vinorelbine; topoisomerase inhibitors such as doxorubicin, daunorubicin (daunomycin), dactinomycin, teniposide, epirubicin, etoposide (VP-16), idarubicin, irinotecan,

mitoxantrone, topotecan, razoxane, sobuzoxane and 9-nitrocamptothecin;androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, and testolactone; anti-adrenals such as aminoglutethimide, mitotane, and trilostane; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; dacarbazine; demecolcine; diaziquone; difluoromethylornithine (DMFO); edatrexate; eflornithine; elliptinium acetate; etoglucid; 2-ethylhydrazide; gacytosine; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitobronitol; mitolactol; mitoguazone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; procarbazine; polysaccharide-K (PSK); sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; urethan; pipobroman; ibandronate; retinoic acid; and inhibitors of receptor tyrosine kinases and/or angiogenesis, including sorafenib (NEXAVAR®), Sunitinib (SUTENT®), pazopanib (VOTRIENT™), toceranib (PALLADIA™), vandetanib (ZACTIMA™, cediranib (RECENT3N®), regorafenib (BAY 73-4506), axitinib (AG013736), lestaurtinib (CEP-701), erlotinib (TARCEVA®), gefitinib (IRESSA™), Afatinib (TOVOK™), lapatinib (TYKERB®), neratinib (HKI-272); PARP inhibitors such as olaparib, and the like, and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[00102] In a specific embodiment, the chemotherapeutic agent is paclitaxel, a PARP inhibitor, a platinum agent, or doxorubicin.

[00103] In some embodiments, the chemotherapeutic agent in the pharmaceutical composition is paclitaxel.

[00104] In some embodiments, the chemotherapeutic agent in the pharmaceutical composition is a PARP inhibitor or a platinum agent.

[00105] In some embodiments, the PARP inhibitor in the pharmaceutical composition is olaparib, rucaparib, niraparib, talazoparib, veliparib, pamiparib (BGB-290), Iniparib (BSI 201), CEP 9722, E7449, AG-14361, INO-1001, A-966492, PJ34 HCl, UPF 1069, AZD2461, ME0328, BGP-15 2HCl, Niraparib tosylate, NU1025, NVP-TNKS 656, NMS-P118, benzamide or picolinamide, or a pharmaceutically acceptable salt, acid or derivative thereof. In one specific embodiment, the PARP inhibitor is olaparib.

[00106] In some embodiments, the platinum agent in the pharmaceutical composition is carboplatin, cis-platin, oxaliplatin, lobaplatin, enloplatin or miboplatin, or a pharmaceutically acceptable salt, acid or derivative thereof. In one specific embodiment, the platinum agent is carboplatin. In one specific embodiment, the platinum agent is cis-platin.

[00107] In some embodiments, the chemotherapeutic agent in the pharmaceutical composition is docetaxel, albumin bound paclitaxel, altretamine, capecitabine, cyclophosphamide, dactinomycin, etoposide, gemcitabine, ifosfamide, irinotecan, liposomal doxorubicin, melphalan, pemetrexed, topotecan, vinblastine, vincristine or vinorelbine, or a pharmaceutically acceptable salt, acid or derivative thereof.

[00108] In some embodiments, the anti-cancer agent in the pharmaceutical composition is an anti-hormonal agent. The anti-hormonal agent may be anti-estrogen or selective estrogen receptor modulator such as tamoxifen, raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene; an aromatase inhibitor such as letrozole, anastrozole, and exemestane; a luteinizing-hormone-releasing hormone (LHRH) agonist such as goserelin, leuprolide and triptorelin; or an anti-androgen such as flutamide, nilutamide, bicalutamide, leuprohde, and goserelin; or a pharmaceutically acceptable salt, acid or derivative thereof.

[00109] In some embodiments, the anti-cancer agent in the pharmaceutical composition is a therapeutic antibody. The therapeutic antibody may be 3F8, abagovomab, adecatumumab, afutuzumab, alemtuzumab, altumomab, amatuximab, anatumomab, arcitumomab, bavituximab, bectumomab, bevacizumab, bivatuzumab, blinatumomab, brentuximab, cantuzumab, catumaxomab, CC49, cetuximab, citatuzumab, cixutumumab, clivatuzumab, conatumumab, dacetuzumab, dalotuzumab, daratumumab, detumomab, drozitumab, duligotumab, dusigitumab, ecromeximab, elotuzumab, ensituximab, ertumaxomab, etaracizumab, farletuzumab, ficlatuzumab, figitumumab, flanvotumab, futuximab, ganitumab, gemtuzumab, girentuximab, glembatumumab, ibritumomab, igovomab, imgatuzumab, indatuximab, inotuzumab, intetumumab, ipilimumab (YERVOY®, MDX-010, BMS-734016 and MDX-101), iratumumab, labetuzumab, lexatumumab, lintuzumab, lorvotuzumab, lucatumumab, mapatumumab, matuzumab, milatuzumab, minretumomab, mitumomab, moxetumomab, naptumomab, narnatumab, necitumumab, nimotuzumab, nofetumomab, obinutuzumab, ocaratuzumab, ofatumumab, olaratumab, onartuzumab, oportuzumab, oregovomab, panitumumab, parsatuzumab, patritumab, pemtumomab, pertuzumab, pintumomab, pritumumab, racotumomab, radretumab, rilotumumab, rituximab, robatumumab, satumomab, sibrotuzumab, siltuximab, simtuzumab, solitomab, tacatuzumab, taplitumomab, tenatumomab, teprotumumab, tigatuzumab, tositumomab, trastuzumab, tucotuzumab, ublituximab, veltuzumab, vorsetuzumab, votumumab, or zalutumumab, or a variant or a fragment thereof.

[00110] In some embodiments, the anti-cancer agent in the pharmaceutical composition is an angiogenesis inhibitor. The angiogenesis inhibitor may be axitinib, bevacizumab, cabozantinib, everolimus, lenalidomide, lenvatinib mesylate, pazopanib, ramucirumab, regorafenib, sorafenib, sunitinib, thalidomide, vandetanib, or ziv-aflibercept, or a pharmaceutically acceptable salt, acid or derivative thereof.

[00111] The pharmaceutical composition of the present disclosure may be prepared in a suitable dosage form, including, but not limited to, tablets, capsules (e.g., soft elastic gelatin capsules), caplets, troches, lozenges, dispersions, suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions),

suppositories, ointments, cataplasms (poultices), pastes, powders, dressings, creams, plasters, solutions, elixirs, cachets, aerosols (e.g., nasal sprays or inhalers), gels, and patches.

[00112] In various embodiments, the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers. The carrier can take a wide variety of forms depending on the form of preparation desired for administration. The carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Alternatively, the carrier can be a solid dosage form carrier, including but not limited to one or more of a binder (for compressed pills), a glidant, an encapsulating agent, a flavorant, and a colorant. Suitable pharmaceutical carriers described in "Remington's Pharmaceutical Sciences" by E.W. Martin are also contemplated by the present disclosure.

[00113] In some embodiments, the pharmaceutical composition is formulated into an oral dosage form. Suitable oral dosage forms include but are not limited to dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups) and the like. In preparing the compositions for an oral dosage form, any of the usual pharmaceutical media can be employed as carriers, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like in the case of oral liquid preparations (such as suspensions, solutions, and elixirs) or aerosols; or carriers such as starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents can be used in the case of oral solid preparations.

EXAMPLES

[00114] The present invention is also described and demonstrated by way of the following examples. However, the use of these and other examples anywhere in the specification is illustrative only and in no way limits the scope and meaning of the invention or of any exemplified term. Likewise, the invention is not limited to any particular preferred embodiments described here. Indeed, many modifications and variations of the invention may be apparent to those skilled in the art upon reading this specification, and such variations can be made without departing from the invention in spirit or in scope. The invention is therefore to be limited only by the terms of the appended claims along with the full scope of equivalents to which those claims are entitled.

EXAMPLE 1: Identification of synthetic lethality of sibutramine with KRAS variant cells and tumors

[00115] To identify drugs that exhibit synthetic lethality with KRAS variant ovarian cancer cells, SKOV3 ovarian cancer cells and isogenic cells harboring the KRAS-variant (22-3 cells) were screened against the Enzo FDA and NCI Oncology libraries at the Yale Center for Molecular Discovery. As predicted, cells harboring the KRAS-variant were significantly more resistant to platinum drugs than isogenic controls not having this variant. However, KRAS-variant ovarian cancer cells were highly sensitive to sibutramine, a drug previously approved by the FDA to promote weight loss. A follow-up clonogenic survival assay was performed wherein the SKOV3 ovarian cancer cells and isogenic cells harboring the KRAS-variant (22-3 cells) were incubated with an increasing concentration of sibutramine (0-200μM) and measured for percent survival at the end of the experiment. The clonogenic survival assay was conducted by plating SKOV3 and 22-3 cells at various densities, treating with various concentrations of sibutramine, removing the sibutramine after 2 days, and replacing with cell growth medium. Colonies were then allowed to form for 12-14 days, stained with crystal violet, and counted. The results are shown in FIG. 2. The data provide strong evidence that 22-3 cells harboring the KRAS-variant exhibit significantly greater sensitivity to sibutramine than parental isogenic controls.

[00116] The effects of sibutramine were also examined in a tumor xenograft study. In a pilot study using 2-3 mice per treatment group, 5×10^6 cells were implanted subcutaneously into athymic nude mice, allowed to grow to $100mm^3$, and treated intraperitoneally with 30 mg/kg of sibutramine daily for 6 weeks. Tumor size and body weight of mice were measured. Sibutramine treatment had no effect on the growth of the SKOV3 xenograft with wild type KRAS (FIG. 3A). In contrast, sibutramine treatment resulted in considerable suppression of tumor growth of the 22-3 xenograft harboring the KRAS-variant (FIG. 3B). The 6-week sibutramine treatment was well tolerated by the mice. Mice regained weight upon cessation of sibutramine treatment. These data demonstrate that sibutramine kills KRAS-variant ovarian cancer cells and ovarian cancer xenografts and suggests that it can be repurposed to treat KRAS-variant ovarian cancer.

EXAMPLE 2: Evaluation of sibutramine alone and in combination with platinum and PARP inhibitor therapies in KRAS-variant ovarian cancer cells and xenografts in mice

[00117] Sibutramine is tested for synergistic, additive, or antagonistic effects for the combination of either carboplatin, paclitaxel, or olaparib and sibutramine, based on the median effect principle (MEP) of Chou and Talaly (10, 11). These experiments are first performed in multi-well plates in which both the anti-neoplastic drug concentrations and

sibutramine concentrations are varied to provide the data set needed to query for synergism. Cell responses to the drugs are measured by quantifying cell proliferation in a microplate format, using the Cytation 3 Imaging Reader (BioTek, Inc.), which can perform live cell, automated fluorescence microscopy in 96- and 384-well microplates. The imaging compartment is temperature- and CO_2-regulated for live cell imaging. In addition, the Cytation 3 has built-in spot recognition software for automatic cell counting, using a process referred to as cell segmentation (28, 29). The viable nuclear dye, Hoechst 33342 (H33342), is used in these experiments (30). This approach facilitates accurate cell enumeration by the Cytation 3 spot recognition algorithms, especially at high cell densities (28, 29). This approach is chosen because it can be readily applied to a wide range of diverse cell lines, without the requirement for extensive optimization initially for each cell line, and does not rely on cellular metabolism as with other types of assays including cell titer glo and MTT. Cell numbers can be measured in a robust and highly reproducible manner, at multiple time-points and cell seeding densities for the SKOV and SKOV 22-3 cells and a variety of other cell lines. Using this method, combination index (CI) values are determined, with values < 1, = 1, and > 1 indicative of synergism, additive effect, and antagonism, respectively. Clonogenic assays are then performed with combinations in which synergism is observed. The most successful therapeutic combinations for studies are then evaluated *in vivo*.

[00118] To confirm that the cytotoxicity observed in the SKOV 22-3 ovarian cancer cells is due to the presence of the KRAS variant, a second isogenic pair of ovarian cancer cell lines, IGROV1-KRAS-variant and IGROV1-wild type (WT) having or not having the KRAS variant, respectively, are utilized. Methods of CRISPR-based single base editing are used to "mutate" the single point mutation in the KRAS 3'UTR (G) of IGROV1 cells back to the wild-type base (T) to generate an isogenic cell line not harboring the KRAS variant. Clonal cells that are confirmed to have the wild-type base are used as controls in experiments to determine if sibutramine preferentially kills KRAS variant IGROV1 cells, using methods described for SKOV cells. After confirming KRAS variant IGROV1 cells are sensitive to sibutramine, experiments are performed to determine if specific drug combinations (i.e., carboplatin, paclitaxel, or olaparib and sibutramine) act in a synergistic manner, using the IGROV1 KRAS variant cells and the isogenic controls. In the event that CRISPR-based methods are unsuccessful to engineer the "WT" 3'UTR in IGROV1 cells, zinc finger technology is utilized.

[00119] Selected key results are validated in an *in vivo* mice model implanted with human IGROV1 KRAS variant ovarian xenografts or isogenic controls and the responsiveness of the xenografts to the treatments are studied.

[00120] The effect of single agent sibutramine treatment is further examined in SKOV3 and IGROV1 cells. In addition, the dose of sibutramine is varied using the SKOV and

SKOV22-3 cells carrying the KRAS variant to determine the minimum effective dose. First, the cell lines are luciferized using well-established methods (for example see Drapkin (12)). Once stable cell lines express the fluorescent marker, the clonogenic survival assays are performed to confirm that the KRAS variant cells exhibit significantly greater cytotoxicity to sibutramine versus isogenic controls lacking the KRAS variant. An orthotopic model is then employed to assess sibutramine efficacy. Injection of cells into the orthotopic site, in this case, the ovarian bursa, generally reproduces much more of the original tumor microenvironment than injection into the flanks of the mice (27). Female athymic BALB/c nude mice ages 8-11 weeks are used. Tumors are established in the ovarian bursa of mice as described (13) from exponentially growing cultures (numbers of cells are adjusted as necessary for each cell line). Tumor growth is monitored weekly starting 3-4 days after implantation by bioluminescence imaging using the IVIS Spectrum In Vivo Imaging System as described (14). Mice are monitored 2-3 times per week and weighed weekly. Tumor bioluminescence among the various groups is compared using analysis of variance (ANOVA) as described (12,14) to determine if sibutramine induces significantly greater tumor growth delay in KRAS variant versus isogenic control xenografts. Tumor growth is monitored for up to 150 days unless mice exhibit symptoms of being moribund or have weight gain in excess of 30% of body weight due to tumor growth. This experiment consists of eight groups of 20 mice each: (1) mock treated control; (2) 7.5 mg/kg sibutramine-treated control; (3) 15 mg/kg sibutramine-treated control; (4) 30 mg/kg sibutramine-treated control; (5) mock-treated KRAS variant tumors; (6) 7.5 mg/kg sibutramine-treated KRAS variant tumors; (7) 15 mg/kg sibutramine-treated KRAS variant tumors; and (8) 30 mg/kg sibutramine-treated KRAS variant tumors. Twenty mice per experimental group have the power of 80% to detect a difference of 1.25 standard deviations between two groups at 5% significance level. Treatment is administered intraperitoneally as described in Example 1. Tumors are also paraffin-embedded and sectioned for H&E staining and immunohistochemistry staining with anti-Ki-67 antibodies for impaired cell proliferation. The minimum dose of sibutramine needed to impart tumor growth delay is determined from the studies.

[00121] Similar experiments are conducted with the one or two best combinations of sibutramine plus carboplatin, paclitaxel or olaparib, selected based on the findings in cell culture experiments. A dose of sibutramine that induces 50% tumor growth delay (TGD) of SKOV3-KRAS variant tumors is used in combination with carboplatin or olaparib. Eight groups of 20 mice each are used in these experiments: (1) mock treated control; (2) sibutramine-treated control at a dose of 50% TGD; (3) sibutramine-treated control at a dose of 50% TGD plus either carboplatin, paclitaxel or olaparib; (4) control with either carboplatin, paclitaxel, or olaparib; groups 5-8 are with KRAS variant cells but with drugs as described for control tumors. Mice receive intraperitoneal injection once daily with sibutramine

(50% tumor inhibitory dose/kg), olaparib (50 mg/kg), or intravenous injection of taxol (20 mg/kg) as described (15), or once intraperitoneal injection bi-weekly with carboplatin (25 mg/kg) for a 6-week period. Tumor size and body weight, and analysis are performed as described in the experiments with sibutramine alone treatment. The response of the tumors to the combination treatment is then determined. Alternatively, the assays with xenografts as described in Example 1 are performed in the event that the ovarian orthotopic mouse model does not work well. Adverse results for the mice with the combination studies are not anticipated, given that sibutramine is well-tolerated. However, if adverse effects are observed, the experiments with lower doses of the chemotherapy agents based on pilot studies are performed. Follow-up studies are performed with patient-derived xenograft (PDX) models.

EXAMPLE 3: Enantiomers separation and target investigation

[00122] Sibutramine is a racemic mixture (RS) containing two equal forms of the R and S enantiomers. First, both enantiomers of sibutramine are separated and their relative efficacy against the isogenic KRAS-variant 22-3 and SKOV parental ovarian cancer cells is determined as described in Example 2. Next, the ovarian orthotopic tumor assay is used with the same cells as described in Example 2 but with three different doses of the enantiomer that are determined in the cell survival assays. This constitutes a first step in target investigation. The separation of the enantiomers of sibutramine, as well as its metabolites, is performed by chiral stationary phase chromatography as described in (17). The assignment of absolute conformations is known. Second, both metabolites of sibutramine, i.e., desmethylsibutramine (M1) and didesmethylsibutramine (M2), are prepared to determine if these have any efficacy in the cellular and animal models described in Example 2. The chemical structures of sibutramine, desmethylsibutramine and didesmethylsibutramine are shown in FIGs. 5A-5C, respectively. The pure enantiomer metabolites of M1 and M2 are prepared via a racemic synthesis and a chiral separation is conducted. Alternatively, a chiral synthesis is conducted in the event that chiral separation cannot be satisfactorily achieved. Demonstration of differentiation of enantiomers helps determine the structure-activity relationship and allows for the development of more potent compounds for use in animal models and ultimately humans.

EXAMPLE 4: Investigation of the mechanism of action of sibutramine in the KRAS variant cells

[00123] Initial experiments are carried out to provide molecular insights into how sibutramine functions to kill KRAS variant cells. Potential targets of sibutramine action include the serotonin transporter (SERT), miRNA processing machinery and/or RAS signaling.

[00124] In one set of experiments, sibutramine is tested to see if it induces upregulation of the let-7 family of miRNAs, resulting in suppression of KRAS. It is hypothesized that sibutramine may target a protein or proteins that function to degrade let-7 miRNAs, resulting in their upregulation and suppression of expression of their target genes, including KRAS. To test this hypothesis, the levels of the mature let-7a, let-7b, let-7d, and let-7g along with mRNA levels of KRAS are quantified in SKOV WT and SKOV 22-3 KRAS variant ovarian cancer cells that are treated or not with the active compound of sibutramine using the miScript PCR system. This is performed as a function of time after treatment with sibutramine with a range of doses between 10-100 μM for 24 hours and harvested and analyzed at various intervals between 1-24 hours. Similar experiments are performed with the active R or S-metabolites purified and identified in Example 3. The basal levels of let-7 miRNAs may be lower in KRAS variant versus control cells, given previous results in lung tumor cells (2). Without wishing to be bound by theory, any increase in the levels of let-7 miRNAs and any decrease in KRAS mRNA, after treatment with sibutramine in KRAS variant versus control cells, might indicate that sibutramine (or its active compound) is targeting some aspect of miRNA processing.

[00125] The luciferase reporter constructs are used with and without the 3'UTR mutation, as described (2), to confirm that treatment with sibutramine results in upregulation (less degradation) of the let-7 miRNAs, resulting in suppression of KRAS expression. In a follow-up experiment, let-7 miRNAs are overexpressed, one at a time, in KRAS variant and control ovarian cancer cell lines to determine if this leads to a greater change in resistance of KRAS variant versus control cells to sibutramine as well as downregulation of KRAS expression using the luc-reporters. Without wishing to be bound by theory, if let-7a, let-7b, let-7d, and let-7g are upregulated in cells treated with the compound, sibutramine might be targeting a transcription factor or otherwise preventing the miRNA processing machinery from degrading let-7 miRNAs. The concentrations of sibutramine and times of treatment or harvesting are varied based upon initial results.

[00126] Sibutramine-targeted genes are identified as follows. It has been suggested that KRAS gene expression is upregulated in KRAS variant cells. This was originally based on analysis of expression of a luciferase (luc) reporter linked to the 3'UTR of KRAS (2). Specifically, introduction of the luc reporter with the KRAS variant 3'UTR into A549 cells, which express let-7 miRNAs, resulted in a significant increase in the level of luc compared to a WT control. Upregulation of KRAS expression has also been detected by qRT-PCR in the endometria of women and in lung tissues harboring the KRAS variant versus controls (2, 37). Therefore these data suggest that in cells expressing let-7 miRNAs, the presence of the KRAS variant leads to upregulation of KRAS.

[00127] To determine if KRAS is overexpressed in the SKOV22-3 versus SKOV3 lines, quantitative western blotting experiments are first performed as described (38, 39). Levels of activated Ras in cells are then quantified using selective pulldown with the glutathione S-transferase-Raf1-Rad binding domain fusion protein and detection with the EZ detect Ras activation kit as described (38, 39). Quantitative western blotting for phosphorylated ERK, p38 Map Kinase, and Jun are then performed as described (40). These experiments are performed in the presence and absence of various concentrations of sibutramine, and its metabolites. These experiments can also be performed in the presence or absence of various concentrations of any one of fluoxetine, citalopram, escitalopram, sertraline, norsertraline, fluvoxamine, femoxetine, indalpine, alaproclate, cericlamine, ifoxetine, zimelidine, dapoxetine, etoperidone, desmethylcitalopram, didesmethylcitalopram, or seproxetine, or any metabolites thereof. If activated Ras levels are higher in KRAS variant (SKOV22-3) versus control (SKOV3) cells, treatment of the cells with sibutramine decreases RAS activation, which would be consistent with their sensitivity to this drug, given that activated RAS promotes growth. This type of result may indicate that the target(s) of sibutramine is related to KRAS signaling.

[00128] Follow up studies are performed as follows. It has recently been demonstrated that fluoxetine (Prozac), a well-known selective serotonin reuptake inhibitor (SSRI), reduces acinar-to-ductal metaplasia that plays a key role in the development of pancreatic ductal adenocarcinoma (PDAC). Fluoxetine also reduces proliferation of pancreatic cancer cells by reducing intracellular 5-HT (serotonin) concentrations (41), via inhibition of the serotonin transporter SERT. Because 5-HT can bind to Rac and reduce GTP hydrolysis so as to prolong its activation, inhibition of transport into cells could result in reduced levels of 5-HT, less Rac activation, and fewer proliferation signals (41), especially in cells overexpressing KRAS protein.

[00129] Given that sibutramine is a weak SSRI, an additional experiment can be performed to determine if SERT is expressed in the KRAS variant and isogenic control cell lines (including at least SKOV 3 and SKOV22-3), using quantitative western blotting. SERT may be predominantly expressed in KRAS variant versus isogenic controls, or SERT may be expressed in both cell types. If SERT is expressed, a determination as to whether sibutramine and related compounds inhibit 5-HT uptake is made using the neurotransmitter transport uptake assay, which is adaptable to other types of cell lines (Molecular Devices). Specifically, this assay monitors transport using fluorescent dyes with the fluorescence detected using a plate reader. Decreased intracellular fluorescence may be observed in the presence of sibutramine if sibutramine acts to inhibit SERT in KRAS variant cells.

[00130] If SERT is not expressed in KRAS variant cell lines, it is important to note that 5-HT can also be produced in cells and that extracellular 5-HT in serum can bind to

serotonin receptors on the cellular membrane to activate Ras signaling (see for example (42)). Therefore if SERT is not expressed the KRAS variant cells, a determination is made as to whether cell survival is dependent upon intracellular synthesis of 5-HT or transport of 5-HT into cells via receptors. To follow up on intracellular synthesis, expression of Tryptophan hydroxylase 1 (TPH1) in cells is assayed using qRT-PCR (41). Also assayed is whether TPH1 downregulation via an inducible shRNA system, such as TRIPZ, leads to decreased proliferation of the KRAS variant cells (42).

[00131] Assays are also performed to determine which receptors are present in the KRAS variant cells, and whether their downregulation results in decreased proliferation of the KRAS variant cells. If downregulation of either TPH1 or one or more 5-HT receptors results in decreased proliferation of KRAS variant versus control cells, treatment of the downregulated KRAS variant cells (either TPH1 or receptor(s)) with sibutramine would have no effect, and thus be informative of the target. For example, if downregulation of TPH1 leads to preferential decreased proliferation of KRAS variant cells, treatment with sibutramine would not lead to further reductions in proliferation if TPH1 was a direct or indirect target of the drug. While TPH1 and/or receptors might not be the target given what is known about sibutramine, these experiments would be performed if SERT is not expressed in the cells.

EXAMPLE 5: Evaluation of sibutramine treatment in other cancer cell lines

[00132] Sibutramine is tested for its effect in cancer cells other than ovarian cancer. Isogenic pairs of a glioma cancer cell line (U251), a prostate cancer cell line and a breast cancer cell line (MCF7) that have or do not have the KRAS variant are engineered similarly as described for IGROV1 cells. The isogenic pairs are utilized in cell survival assays to determine if the KRAS variant cancer cells are more sensitive to sibutramine treatment.

[00133] Sibutramine in combination with other appropriate chemotherapies are also evaluated in the isogenic pairs of a glioma cancer cell line, a prostate cancer cell line and a breast cancer cell line that have or do not have the KRAS variant. For glioma, sibutramine in combination with an alkylator such as temozolomide is tested. For breast cancer, sibutramine in combination with cisplatin, PARP inhibitor, or paclitaxel, and additionally doxorubicin and/or gemcitabine is tested. The most successful therapeutic combinations for studies are then evaluated in vivo.

[00134] Treatment with sibutramine alone, as well as a combination of sibutramine with other chemotherapies, are evaluated in preclinical mouse xenograft studies with isogenic xenograft pairs. Response of xenograft tumors to the treatments are determined as described in the Example 1.

EXAMPLE 6: Development of sibutramine derivatives

[00135] Based on the enantiomer activities determined in Example 3, molecules that resemble the active enantiomer of sibutramine are designed and developed. These molecules are evaluated in cell-based and biochemical assays and preclinical assays to determine activity. Selected molecules are further refined based on the structure-activity relationships revealed by the activity assays to improve their activities. Additionally, the molecules are also adapted for improved cell permeability. Brain-penetrable analogs are developed for use in KRAS variant glioma.

EXAMPLE 7: Structure-based drug design

[00136] There are two possible targets of sibutramine: the serotonin transporter (SERT) and the XRN2 nuclease. The crystal structures exist for both SERT (PDB: 5I6X) and XRN2 from *C. elegans* (PDB: 5FIR), as shown in FIGs. 4A and 4B, respectively. The crystal structure of SERT is at 3.15 Angstroms and the crystal structure of XRN2 is at 2.84 Angstroms. Predictive algorithms are run on the high-resolution structures to suggest the number and quality of potential binding sites and assess the extent to which the proteins can be targeted by a drug. Information of the binding sites is used to design or modify candidate compounds with improved efficacy.

EXAMPLE 8: Cellular pharmacodynamic studies

[00137] Cellular pharmacodynamic studies were conducted for the effects of fluoxetine (FE), sibutramine (SE), cisplatin (CisP), and paclitaxel (P) on the inhibition of the proliferation of breast cancer cell line MCF-7 (KRAS LCS-6 mutation, LCS-6G below) and ZR-75-1 (LCS-6 wild-type, LCS-6T below), with cisplatin (CisP) and paclitaxel (P) used as the positive control.

Cell culture of MCF-7 and ZR-75-1 cell lines and preparation of blank control

[00138] MCF-7 cells and ZR-75-1 cells were cultured to the logarithmic growth phase with RPMI 1640 medium, digested with trypsin, and then centrifuged at 1000 rpm for 5 minutes to collect the cells. The supernatant was discarded and appropriate amount of medium was added to resuspend the cells. 90 μL of cell suspension was added to each well of a 96-well plate, with a final cell density of 1000-6000 cells/well.

[00139] The T0 plate (i.e., the control plate) was subject to a condition of 37°C, 5% CO_2, and 95% humidity. On the next day, the chemiluminescence of the T0 plate was read as the background value. 10 μL of solvent-containing medium was added to each well for CTG analysis: the CTG reagent was thawed and the cell plate was equilibrated to room

temperature for 30 minutes; 50 µL of CTG solution was added to each well; the plate was incubated on an orbital shaker for 2 minutes to lyse the cells; the cell plate was allowed to sit at room temperature for 10 minutes to stabilize the luminescence signal; and EnVision (2104 Multilabel Reader, PerkinElmer) was used to read the luminescence value. On the same day, the drugs were added, and cisplatin was added as the positive control drug.

Preparation and administration of drug solution

[00140] Ten times concentration drugs were prepared as 10xFE, 10xSE, 10xP, and 10xCisP according to Table 1. 10 µL of 10x drug solution was added to the 90 µL of cell suspension in each well (a triplicate of wells were allocated for each drug concentration) for a final concentration of 1x, and totally 9 different concentrations were used. The cells were cultured under a condition of 37°C and 5% CO_2. After 72 hours of drug treatment, the chemiluminescence of the plate was read: the CTG (cell-titer glo) reagent was thawed and the cell plate was equilibrated to room temperature for 30 minutes; 50 µL CTG solution was added to each well; the plate was vibrated on an orbital shaker for 2 minutes to lyse the cells; the plate was allowed to sit at room temperature for 10 minutes to stabilize the luminescence signal; and EnVision (2104 Multilabel Reader, PerkinElmer) was used to read the luminescence value.

Table 1. Ten Times (10x) Concentration Drugs

FE	1000 µM	500 µM	400 µM	300 µM	200 µM	100 µM	50 µM	25 µM	10 µM
SE	1000 µM	500 µM	400 µM	300 µM	200 µM	100 µM	50 µM	25 µM	10 µM
CisP	1mM	333.3µM	111.1µM	37.04µM	12.35µM	4.11µM	1.37µM	457nM	152nM
P	1000 nM	500 nM	100 nM	80 nM	50 nM	40 nM	30 nM	20 nM	10 nM

Inhibitory effects of FE, SE, CisP, and P drugs on the proliferation of breast cancer cell line MCF7

[00141] The cell viability formula is as follows:

[00142] (Vsample-Vmedium control) / (Vvehicle control-Vmedium control) x 100%

[00143] Vsample is the chemiluminescence of the drug treatment group, and Vvehicle control is the average value for the solvent control group.

[00144] In GraphPad Prism 5.0 software, a non-linear regression model was used to draw the S-shaped dose-viability curve and the IC_{50} value was calculated. The maximum inhibition rate was also calculated. The results are shown in Table 2.

Table 2. IC_{50} Value and Max Inhibition Ratio of Drugs on MCF-7 Cell Line

Cell Line Name	Test Article	Absolute IC_{50}	Max inhibition
MCF-7	SE	35.72μM	99.35%
	FE	16.03μM	99.75%
	Cisplatin	16.91μM	93.55%
	P	26.13nM	55.07%

[00145] Graphs showing the inhibitory effects of fluoxetine (FE), sibutramine (SE), cisplatin (CisP), and paclitaxel (P) on the proliferation of MCF-7 cells, with cisplatin (CisP) and paclitaxel (P) used as the positive control, are displayed in FIGs. 6A and 6B.

Inhibitory effects of FE, SE, CisP and P drugs on the proliferation of breast cancer cell line ZR-75-1

[00146] The cell viability formula is as follows:

[00147] (Vsample-Vmedium control) / (Vvehicle control-Vmedium control) x100%

[00148] Vsample is the chemiluminescence of the drug treatment group, and Vvehicle control is the average value for the solvent control group.

[00149] In GraphPad Prism 5.0 software, a non-linear regression model was used to draw the S-shaped dose-viability curve and the IC_{50} value was calculated. The maximum inhibition rate was also calculated. The results are shown in Table 3.

Table 3. IC_{50} Value and Max Inhibition Ratio of Drugs on ZR-75-1 Cell Line

Cell Line Name	Test Article	Absolute IC_{50}	Max inhibition
ZR-75-1	SE	67.01μM	77.64%
	FE	15.68μM	99.61%
	Cisplatin	15.41μM	93.17%
	P	26.27nM	56.59%

[00150] Graphs showing the inhibitory effects of fluoxetine (FE), sibutramine (SE), cisplatin (CisP), and paclitaxel (P) on the proliferation of ZR-75-1 cells, with cisplatin (CisP) and paclitaxel (P) used as the positive control are shown in FIGs. 7A-7B.

[00151] Based on the results shown in Table 2 and Table 3 and FIGs. 6A-6B and FIGs. 7A-7B, the inhibitory effect of SE on the proliferation of the LCS-6 mutant cells (MCF-7) is significantly stronger than that of LCS-6 wild-type cells (ZR-75-1), while FE, CISP and P drugs show minimal difference in their inhibition on cell proliferation, suggesting that SE drugs act on the mutation site of LCS-6. Moreover, both FE and SE exhibit higher max inhibition as compared to CisP and P drug, indicating their potential use against breast cancer cells.

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* * *

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims. It is further to be understood that all values are approximate, and are provided for description.

Patents, patent applications, publications, product descriptions, and protocols are cited

throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.

CLAIMS

1. A method of treating a cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of a serotonin uptake inhibitor, wherein the subject comprises rs61764370 variant of the KRAS gene.

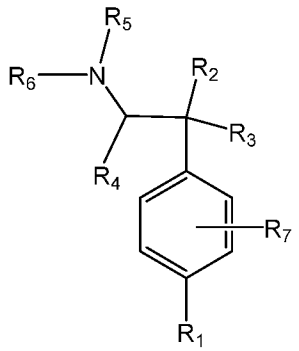
2. The method of claim 1, wherein the cancer is an ovarian cancer.

3. The method of claim 2, wherein the ovarian cancer is a hereditary ovarian cancer.

4. The method of claim 2, wherein the ovarian cancer is an epithelial ovarian cancer.

5. The method of claim 1, wherein the cancer is a breast cancer.

6. The method of any one of claims 1-5, wherein the serotonin uptake inhibitor has a structure according to formula (I):



(I),

wherein R_1 is selected from H, a halide, and a C_1-C_3 perfluorocarbon;

R_2 and R_3 are independently selected from H and a C_1-C_3 hydrocarbon, or R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;

R_4 is selected from H and a C_1-C_6 hydrocarbon optionally substituted with -OH or a halide;

R_5 and R_6 are independently selected from H and a C_1-C_3 hydrocarbon;

R_7 is absent or is selected from a halide and a C_1-C_3 perfluorocarbon,

or a pharmaceutically acceptable salt thereof.

7. The method of claim 6, wherein:

R_1 is selected from a halide and a C_1-C_3 perfluorocarbon;

R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;

R_4 is a C_1-C_6 hydrocarbon optionally substituted with -OH or a halide;

R_5 and R_6 are independently selected from H and -CH_3;

R_7 is absent or is a halide,

or a pharmaceutically acceptable salt thereof.

8. The method of claim 6, wherein:

R_1 is a halide;

R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;

R_4 is a C_3-C_6 hydrocarbon optionally substituted with -OH or a halide;

R_5 and R_6 are independently selected from H and -CH_3;

R_7 is absent,

or a pharmaceutically acceptable salt thereof.

9. The method of claim 6, wherein:

R_1 is -Cl;

R_2 and R_3 together form a 4-membered carbocycle;

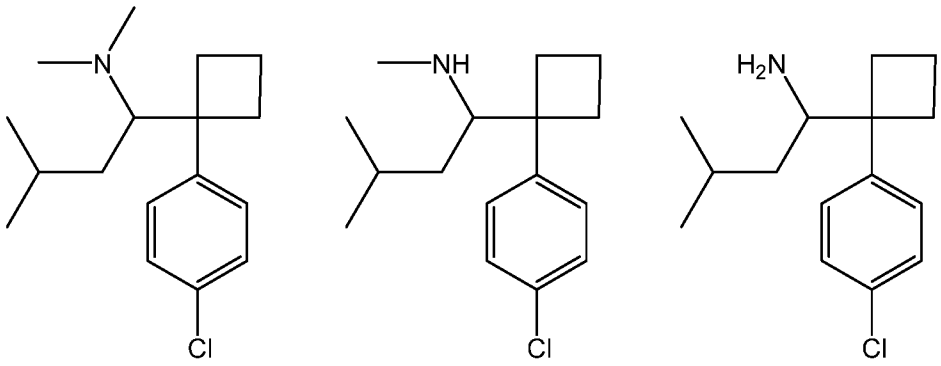
R_4 is a selected from n-propyl, isopropyl, n-butyl, isobutyl, and t-butyl;

R_5 and R_6 are independently selected from H and -CH_3;

R_7 is absent,

or a pharmaceutically acceptable salt thereof.

10. The method of claim 6, wherein the compound of formula (I) is selected from



sibutramine, desmethylsibutramine, and didesmethylsibutramine,

or a pharmaceutically acceptable salt thereof.

11. The method of any one of claims 1-5, wherein the serotonin uptake inhibitor is selected from fluoxetine, citalopram, escitalopram, sertraline, norsertraline, fluvoxamine, femoxetine, indalpine, alaproclate, ericlamine, ifoxetine, zimelidine, dapoxetine, etoperidone, desmethylcitalopram, didesmethylcitalopram, and seproxetine.

12. The method of any one of claims 1-5, wherein the serotonin uptake inhibitor is sibutramine.

13. The method of claim 12, wherein the serotonin uptake inhibitor is (R)-sibutramine.

14. The method of claim 12, wherein the serotonin uptake inhibitor is (S)-sibutramine.

15. The method of any one of claims 1-5, wherein the serotonin uptake inhibitor is desmethylsibutramine or didesmethylsibutramine.

16. The method of claim 15, wherein the serotonin uptake inhibitor is (R)-desmethylsibutramine.

17. The method of claim 15, wherein the serotonin uptake inhibitor is (S)-desmethylsibutramine.

18. The method of claim 15, wherein the serotonin uptake inhibitor is (R)-didesmethylsibutramine.

19. The method of claim 15, wherein the serotonin uptake inhibitor is (S)-didesmethylsibutramine.

20. The method of any one of claims 1-19, wherein the serotonin uptake inhibitor is more effective in killing a cancer cell comprising the rs61764370 variant of the KRAS gene than killing a corresponding cancer cell that does not comprise the rs61764370 variant of the KRAS gene.

21. The method of any one of claims 1-19, wherein the serotonin uptake inhibitor is more effective in inhibiting survival of a cancer cell comprising the rs61764370 variant of the KRAS gene than in inhibiting survival of a corresponding cancer cell that does not comprise the rs61764370 variant of the KRAS gene.

22. The method of any one of claim 1-21, wherein the administration of the serotonin uptake inhibitor results in inhibition of the growth of a tumor of the cancer.

23. The method of any one of claims 1-22, wherein the method further comprises administering one or more additional anti-cancer agents.

24. The method of claim 23, wherein the serotonin uptake inhibitor and the additional anti-cancer agent are administered concurrently.

25. The method of claim 24, wherein the serotonin uptake inhibitor and the additional anti-cancer agent are administered in one composition.

26. The method of claim 23, wherein the serotonin uptake inhibitor and the additional anti-cancer agent are administered sequentially.

27. The method of any one of claims 23-26, wherein the additional anti-cancer agent is a chemotherapeutic agent.

28. The method of claim 27, wherein the chemotherapeutic agent is selected from paclitaxel, a PARP inhibitor, a platinum agent, and doxorubicin.

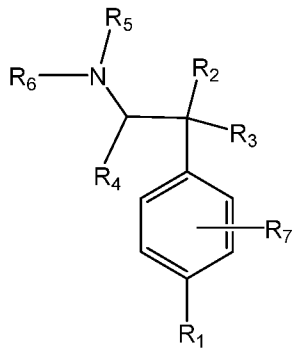
29. The method of any one of claims 1-28, wherein the method further comprises administering to the subject one or more additional anti-cancer treatments selected from a radiation therapy, an immunotherapy and a gene therapy.

30. The method of any one of claims 1-29, wherein the serotonin uptake inhibitor is administered orally.

31. The method of any one of claims 1-30, wherein the subject is a human.

32. A pharmaceutical composition comprising a serotonin uptake inhibitor and one or more additional anti-cancer agents.

33. The pharmaceutical composition of claim 32, wherein the serotonin uptake inhibitor has a structure according to formula (I):



(I),

wherein R_1 is selected from H, a halide, and a C_1-C_3 perfluorocarbon;

R_2 and R_3 are independently selected from H and a C_1-C_3 hydrocarbon, or R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;

R_4 is selected from H and a C_1-C_6 hydrocarbon optionally substituted with -OH or a halide;

R_5 and R_6 are independently selected from H and a C_1-C_3 hydrocarbon;

R_7 is absent or is selected from a halide and a C_1-C_3 perfluorocarbon,

or a pharmaceutically acceptable salt thereof.

34. The pharmaceutical composition of claim 33, wherein:

R_1 is selected from a halide and a C_1-C_3 perfluorocarbon;

R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;

R_4 is a C_1-C_6 hydrocarbon optionally substituted with -OH or a halide;

R_5 and R_6 are independently selected from H and -CH_3;

R_7 is absent or is a halide,

or a pharmaceutically acceptable salt thereof.

35. The pharmaceutical composition of claim 33, wherein:

R_1 is a halide;

R_2 and R_3 together form a 4-, 5-, or 6-membered carbocycle optionally substituted with -OH, a halide, a C_1-C_3 perfluorocarbon, or a C_1-C_3 hydrocarbon;

R_4 is a C_3-C_6 hydrocarbon optionally substituted with -OH or a halide;

R_5 and R_6 are independently selected from H and -CH_3;

R_7 is absent,

or a pharmaceutically acceptable salt thereof.

36. The pharmaceutical composition of claim 33, wherein:

R_1 is -Cl;

R_2 and R_3 together form a 4-membered carbocycle;

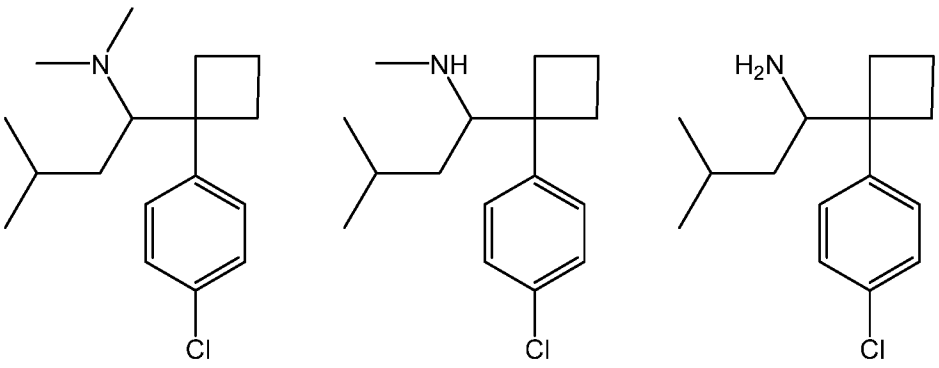
R_4 is a selected from n-propyl, isopropyl, n-butyl, isobutyl, and t-butyl;

R_5 and R_6 are independently selected from H and -CH_3;

R_7 is absent,

or a pharmaceutically acceptable salt thereof.

37. The pharmaceutical composition of claim 33, wherein the compound of formula (I) is selected from



sibutramine, desmethylsibutramine, and didesmethylsibutramine,

or a pharmaceutically acceptable salt thereof.

38. The pharmaceutical composition of claim 32, wherein the serotonin uptake inhibitor is selected from fluoxetine, citalopram, escitalopram, sertraline, norsertraline, fluvoxamine, femoxetine, indalpine, alaproclate, cericlamine, ifoxetine, zimelidine, dapoxetine, etoperidone, desmethylcitalopram, didesmethylcitalopram, and seproxetine.

39. The pharmaceutical composition of claim 32, wherein the serotonin uptake inhibitor is sibutramine.

40. The pharmaceutical composition of claim 39, wherein the serotonin uptake inhibitor is (R)-sibutramine.

41. The pharmaceutical composition of claim 39, wherein the serotonin uptake inhibitor is (S)-sibutramine.

42. The pharmaceutical composition of claim 32, wherein the serotonin uptake inhibitor is desmethylsibutramine or didesmethylsibutramine.

43. The pharmaceutical composition of claim 42, wherein the serotonin uptake inhibitor is (R)-desmethylsibutramine.

44. The pharmaceutical composition of claim 42, wherein the serotonin uptake inhibitor is (S)-desmethylsibutramine.

45. The pharmaceutical composition of claim 42, wherein the serotonin uptake inhibitor is (R)- didesmethylsibutramine.

46. The pharmaceutical composition of claim 42, wherein the serotonin uptake inhibitor is (S)- didesmethylsibutramine.

47. The pharmaceutical composition of any one of claims 32-46, wherein the additional anti-cancer agent is paclitaxel, a PARP inhibitor, a platinum agent, or doxorubicin.

48. The pharmaceutical composition of any one of claims 32-47, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier or excipient.

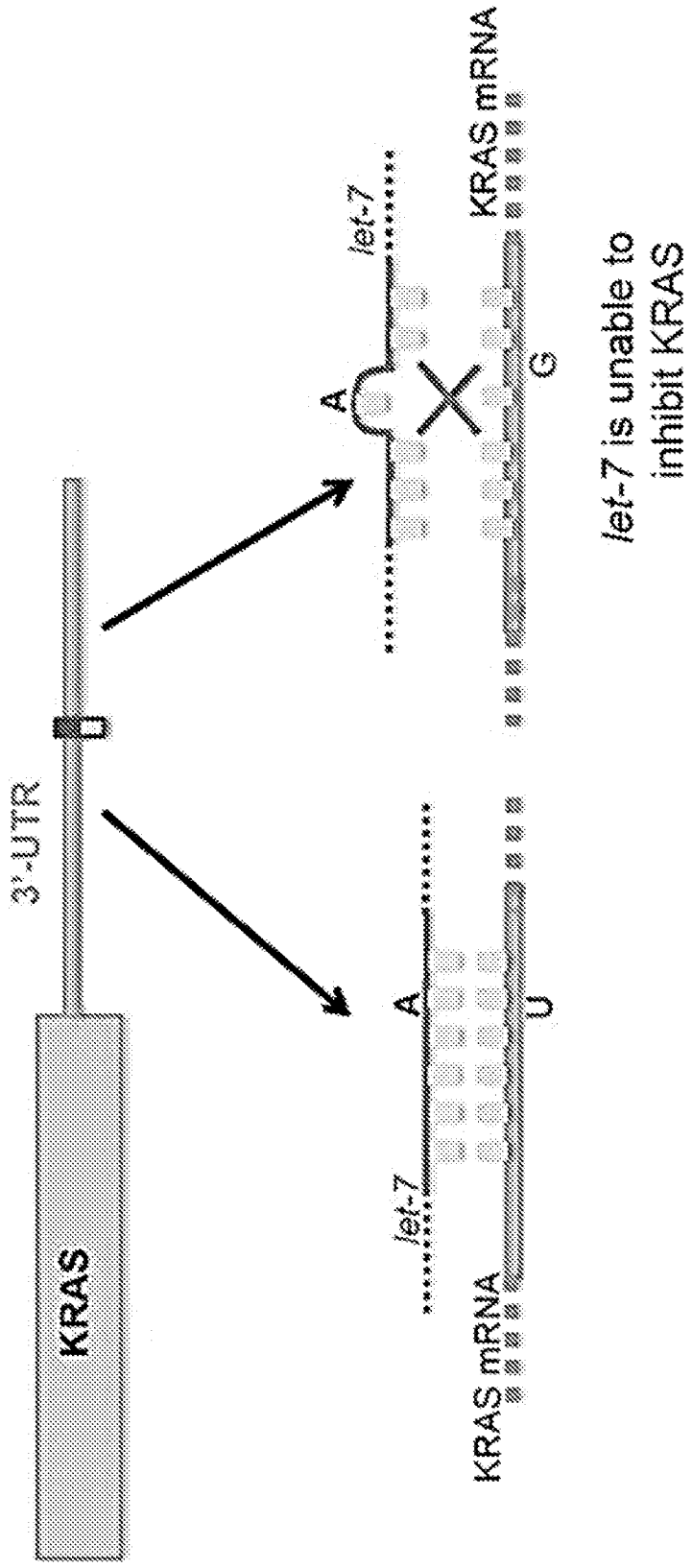

KRAS
3'-UTR
U
let-7
A
KRAS mRNA
U
let-7
A
KRAS mRNA
G
let-7 is unable to inhibit KRAS

FIG. 1

FIG. 2

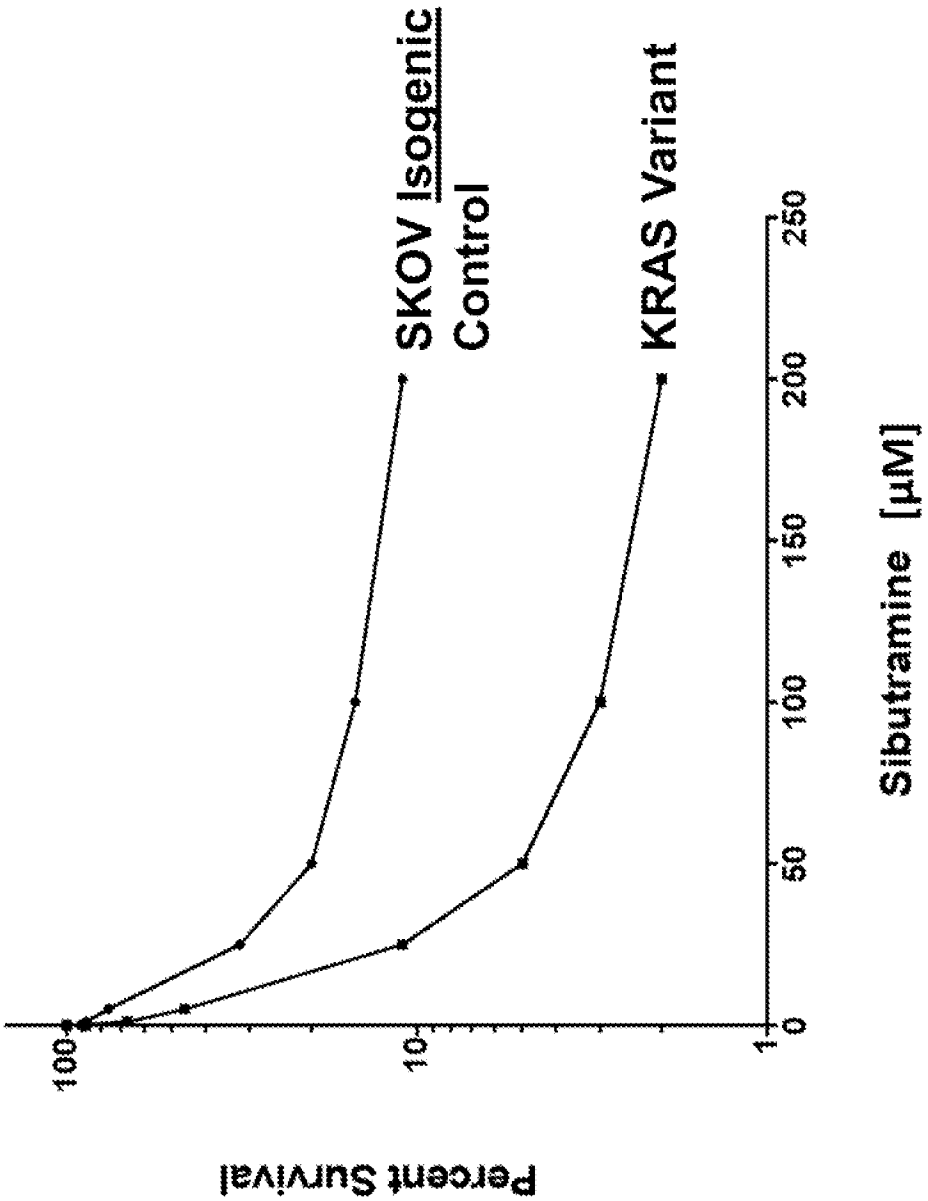

SKOV Isogenic Control
KRAS Variant
Percent Survival
100
10
1
0
50
100
150
200
250
Sibutramine [μM]

FIG. 3A

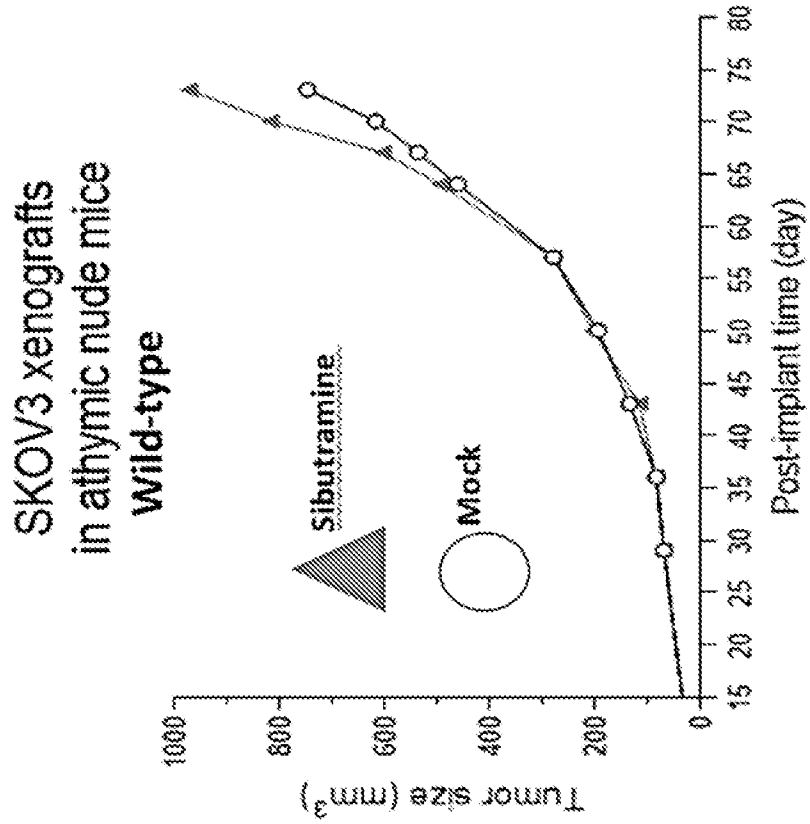
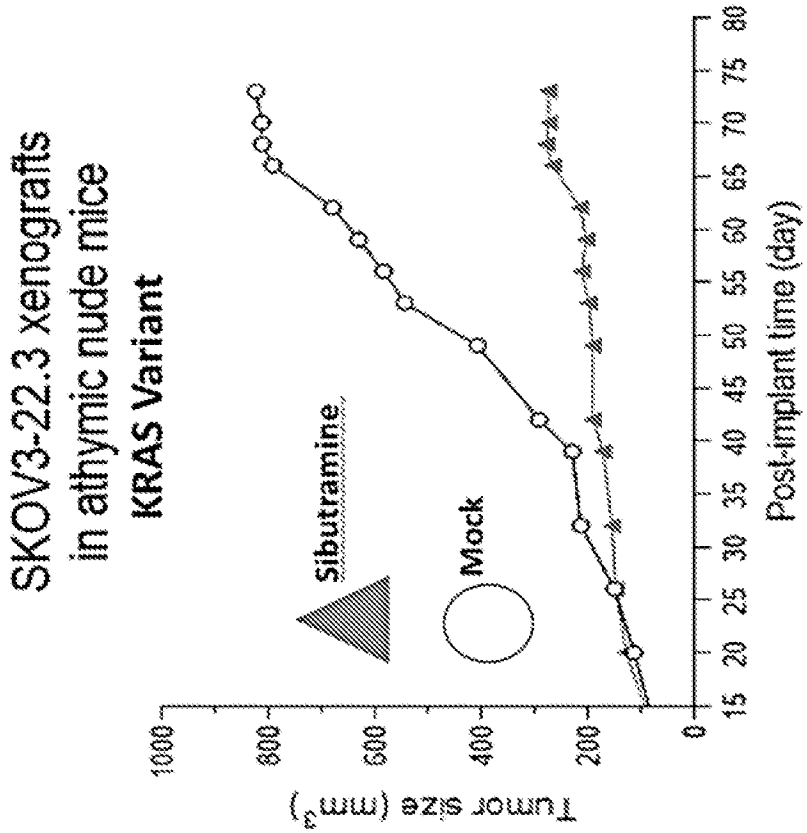
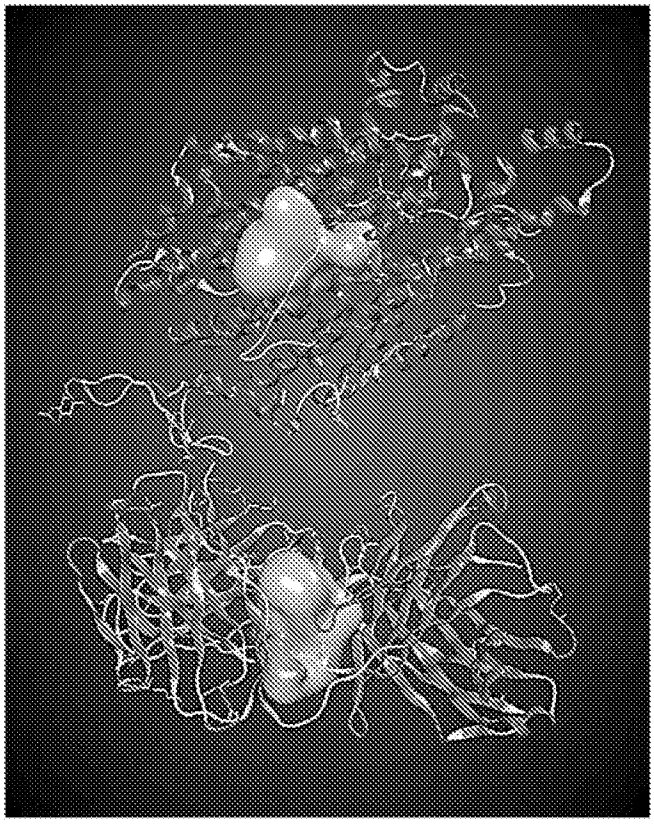

SKOV3 xenografts
in athymic nude mice
Wild-type
Sibutramine
Mock
Tumor size (mm³)
Post-implant time (day)

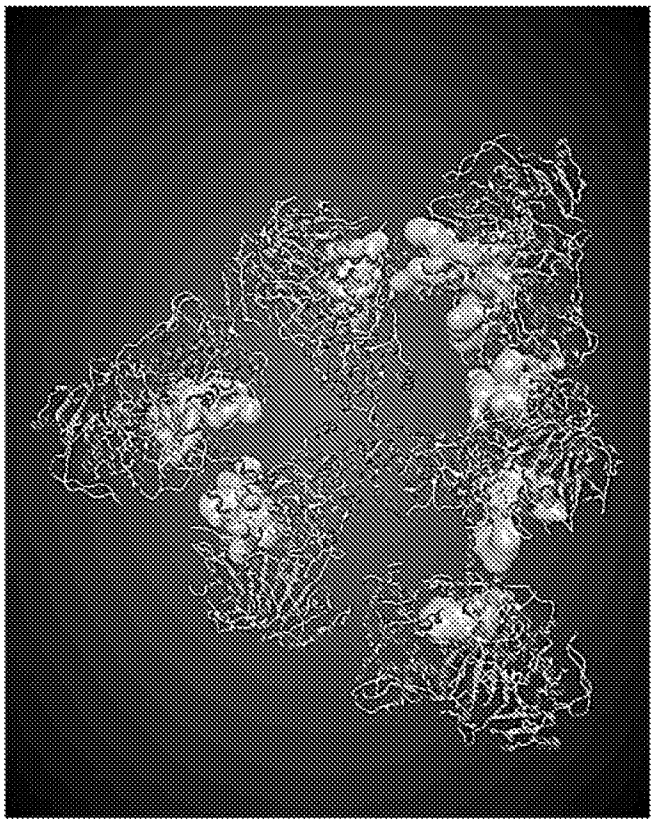
FIG. 3B


SKOV3-22.3 xenografts
in athymic nude mice
KRAS Variant
Sibutramine
Mock
Tumor size (mm³)
Post-implant time (day)



XRN2

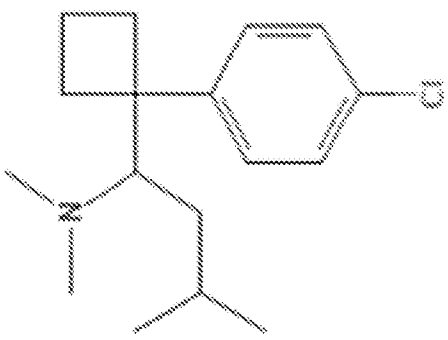
FIG. 4B



SERT

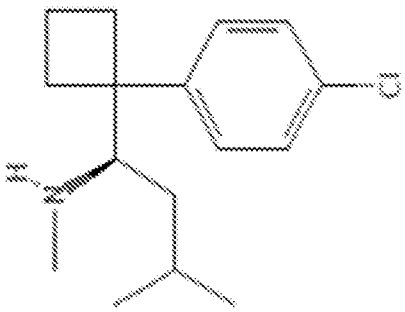
FIG. 4A

FIG. 5A



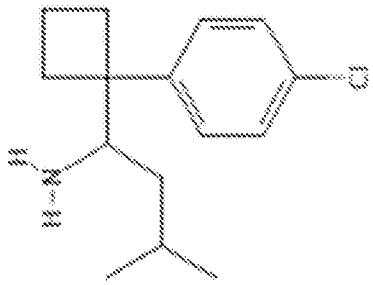
Sibutramine

FIG. 5B



Desmethylsibutramine

FIG. 5C



Didesmethylsibutramine

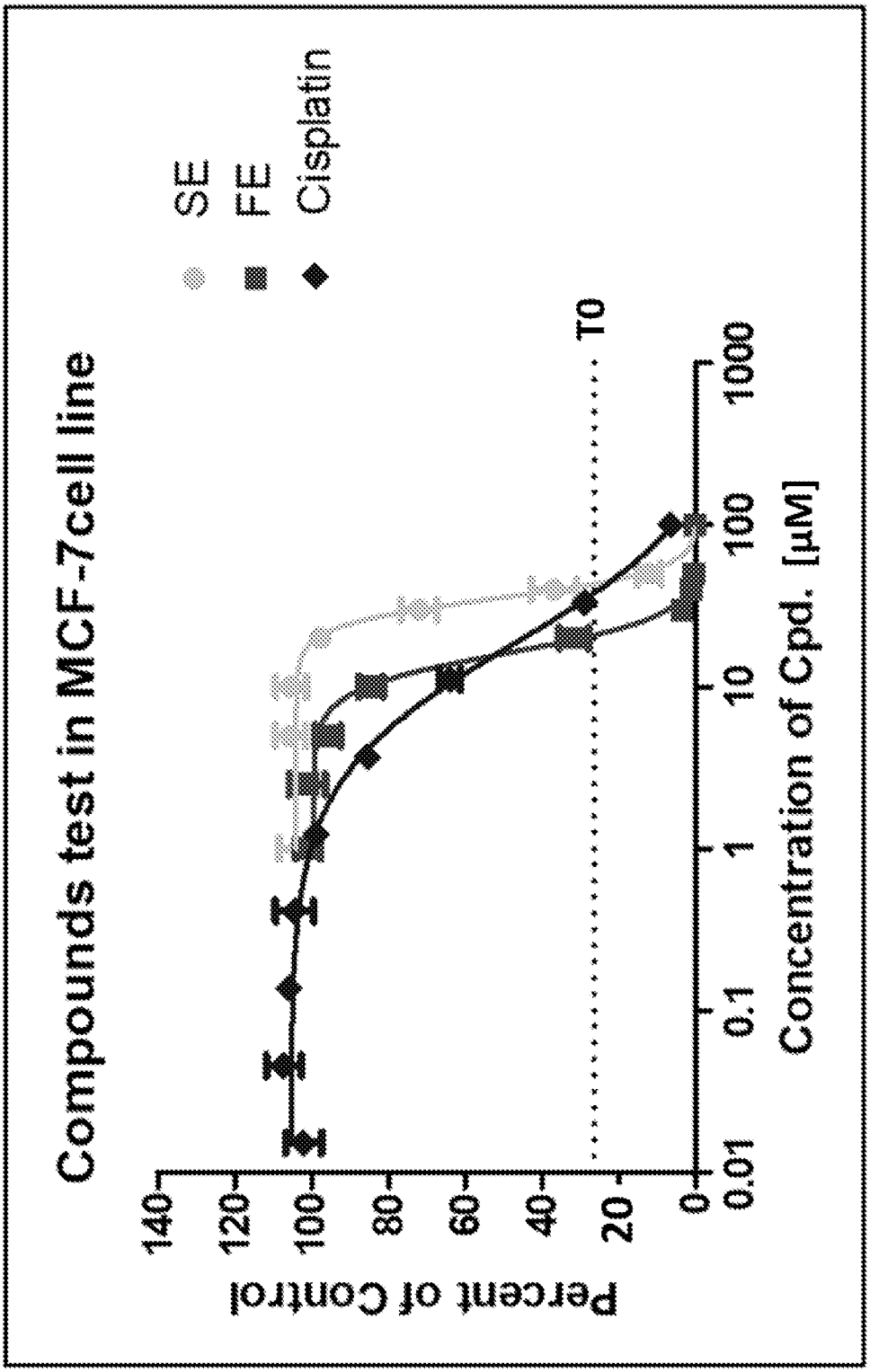

Compounds test in MCF-7cell line
SE
FE
Cisplatin
T0
Percent of Control
140
120
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
Concentration of Cpd. [µM]

FIG. 6A

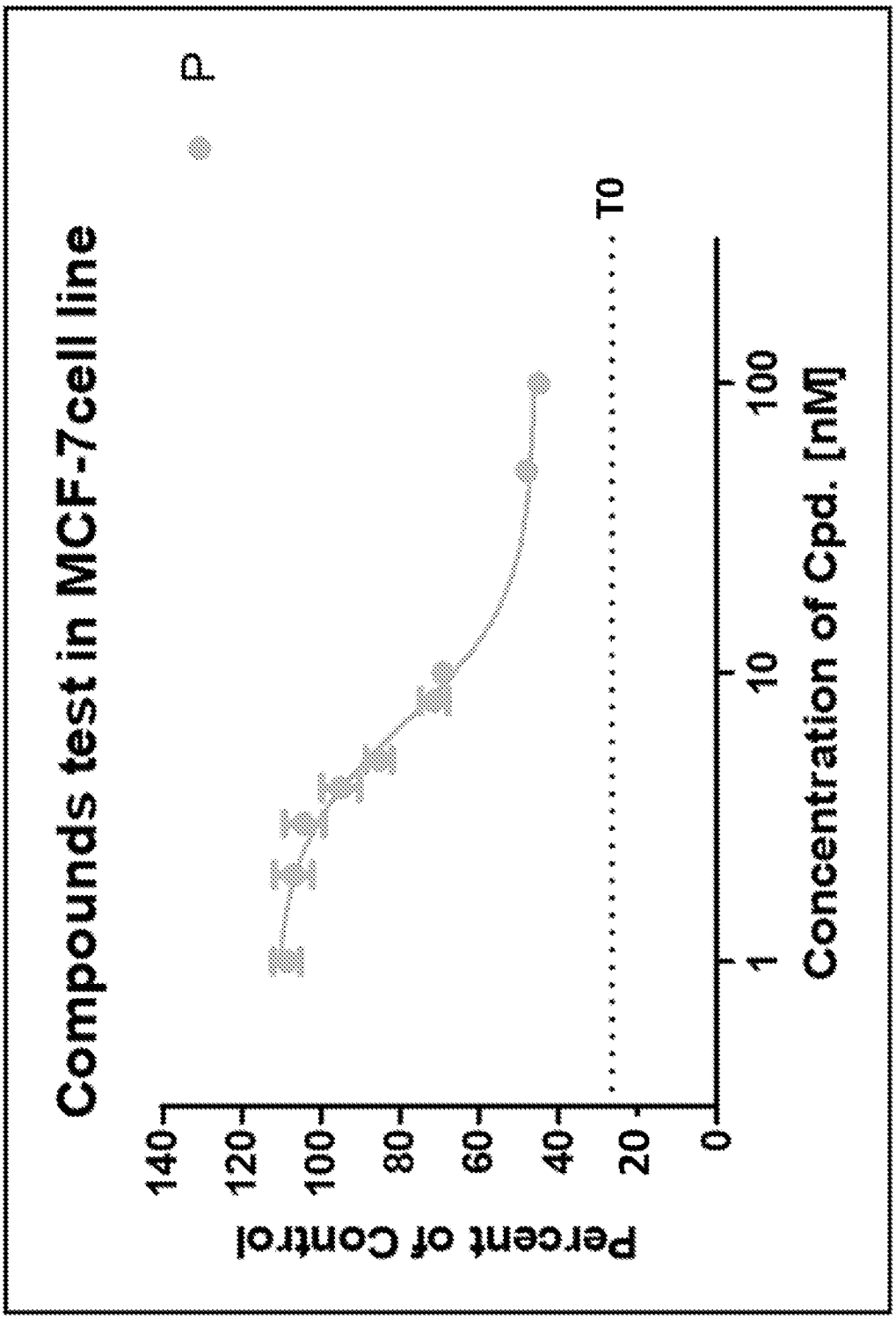

Compounds test in MCF-7cell line
P
T0
Percent of Control
140
120
100
80
60
40
20
0
1
10
100
Concentration of Cpd. [nM]

FIG. 6B

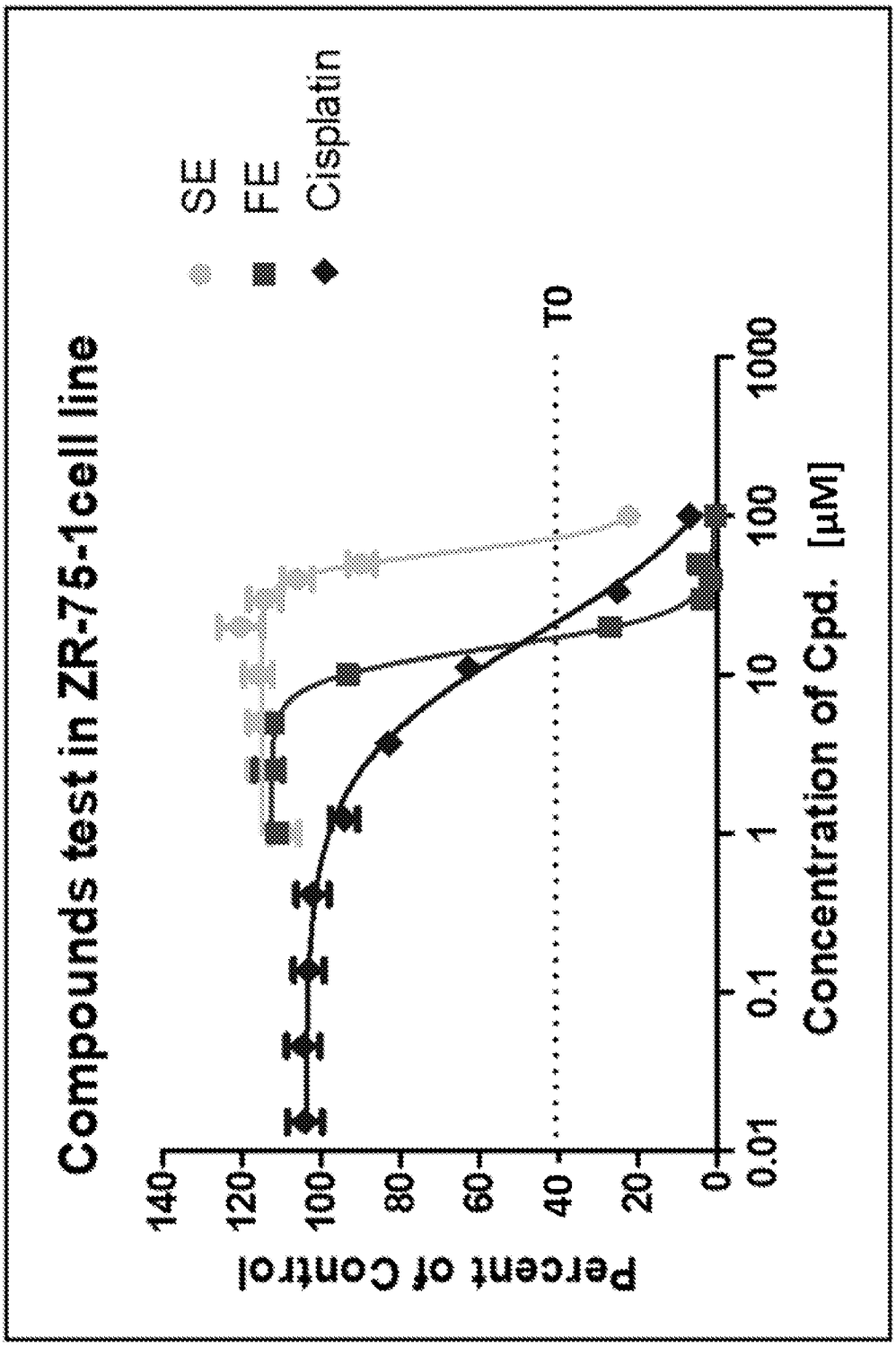

Compounds test in ZR-75-1cell line
SE
FE
Cisplatin
T0
Percent of Control
140
120
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
Concentration of Cpd. [µM]

FIG. 7A

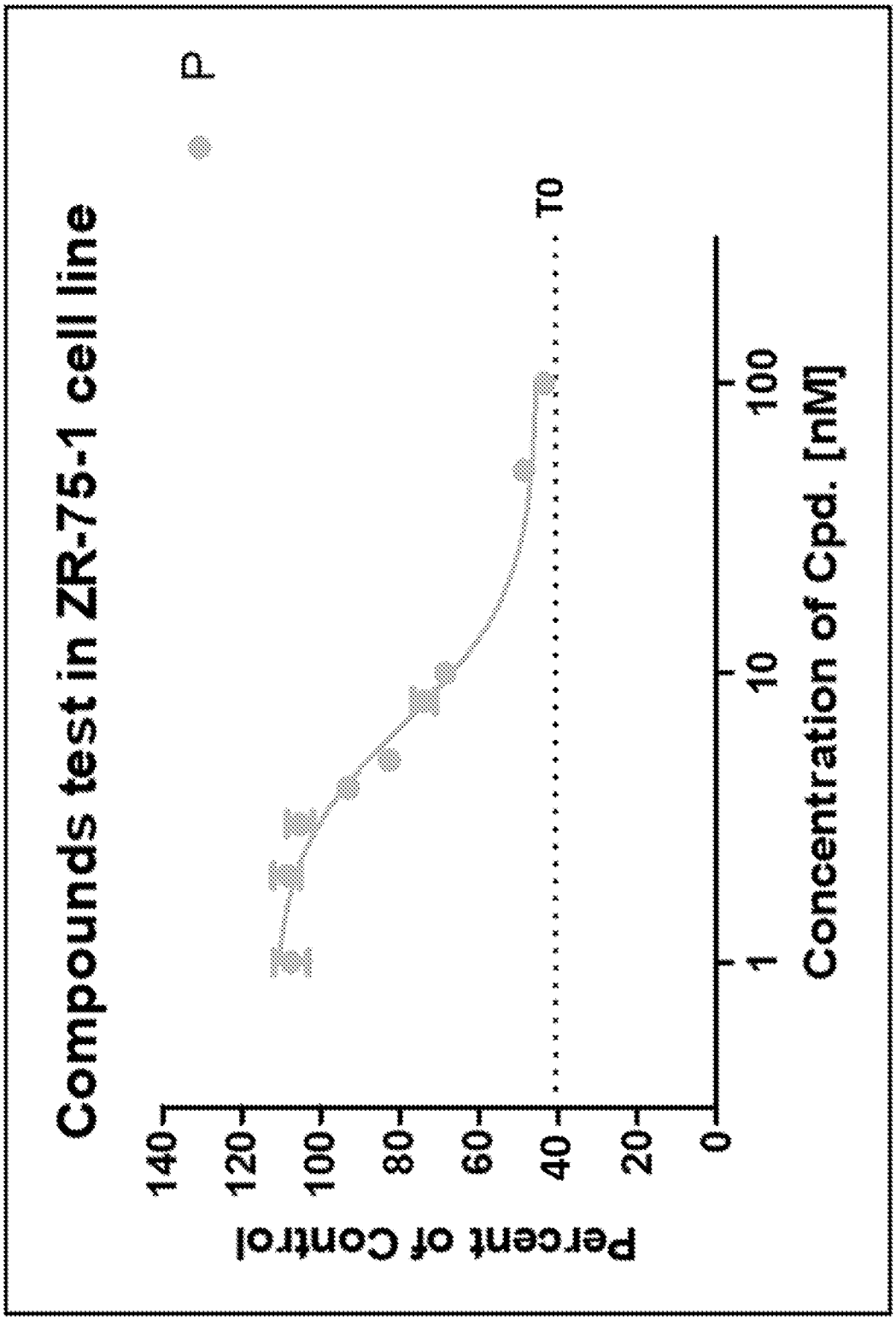

Compounds test in ZR-75-1 cell line
P
T0
Percent of Control
140
120
100
80
60
40
20
0
1
10
100
Concentration of Cpd. [nM]

FIG. 7B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/032715

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/137 (2006.01) ***A61K 31/282 (2006.01)*** ***A61K 31/337 (2006.01)*** ***A61K 31/502 (2006.01)*** ***A61P 35/00 (2006.01)***

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

EPOQUE - Database PATENW, STN - Databases CAPLUS, MEDLINE, BIOSIS, EMBASE and keyword: serotonin uptake, SSRI, sibutramine, KRAS, RS61764370, PARP inhibitor, doxorubicin, cisplatin, paclitaxel, cancer, tumour and related terms

Applicant and Inventor Names searched at Patentscope and internal databases provided by IP Australia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

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

"D" document cited by the applicant in the international application

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 August 2021	17 August 2021
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Leah Walker AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262256170

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SAPONARA, E. et al. "Serotonin uptake is required for Rac1 activation in Kras-induced acinar-to-ductal metaplasia in the pancreas" Journal of Pathology (2018) vol. 246, no. 3, pages 352-365 See page 352 right column paragraph 1 to page 353 left column paragraph 2, page 356 left column paragraph 2 to page 362 left column paragraph 3, page 363 left column final paragraph	32-48
Y	See page 352 right column paragraph 1 to page 353 left column paragraph 2, page 356 left column paragraph 2 to page 362 left column paragraph 3, page 363 left column final paragraph	1-31
X	CN 110743016 A (SUZHOU PRECISION GENE TECH CO LTD) 04 February 2020 & Google Patents translation See Google Patents translation - abstract, claims	32, 38, 47, 48
X	WO 2002/043652 A2 (RAMOT UNIVERSITY AUTHORITY FOR APPLIED RESEARCH & INDUSTRIAL DEVELOPMENT LTD.) 06 June 2002 See claim 60	32, 38, 47, 48
X	US 2004/0054014 A1 (MARGALIT, RIMONA et al.) 18 March 2004 See paragraph [0094], claims 40-47	32, 38, 47, 48
X	US 6974837 B2 (JERUSSI, THOMAS P. et al.) 13 December 2005 See abstract, paragraph bridging columns 4 and 5, claims	32-37, 42-46, 48
X	WO 2002/060427 A2 (SEPRACOR, INC.) 08 August 2002 See abstract, claims	32-37, 39, 40, 48
X	WO 2002/060428 A2 (SEPRACOR, INC.) 08 August 2002 See abstract, claims	32-37, 39, 41, 48
A	PENG, T. et al. "Inhibitors of phosphodiesterase as cancer therapeutics" European Journal of Medicinal Chemistry" (2018) vol. 150, pages 742-756 See whole document in particular Table 2	32-37, 39-46, 48
X	WO 2020/043716 A1 (KATHOLIEKE UNIVERSITEIT LEUVEN) 05 March 2020 See abstract, Figure 14, page 17 line 11 to page 20 line 1, claim 18	32, 38, 47, 48
X	GWYNNE, W. D. et al. "Serotonergic system antagonists target breast tumor initiating cells and synergize with chemotherapy to shrink human breast tumor xenografts" Oncotarget (2017) vol. 8, no. 19, pages 32101-32116 See abstract, page 32102 left column paragraph 2, page 32106 right column last paragraph to page 32110 right column paragraph 2, page 32112 right column paragraph 2	32, 38, 47, 48
Y	WO 2012/129352 A1 (YALE UNIVERSITY) 27 September 2012 See abstract, paragraphs [08]-[10]	1-31

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2021/032715**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
CN 110743016 A	04 February 2020	CN 110743016 A	04 Feb 2020
		CN 112274645 A	29 Jan 2021
WO 2002/043652 A2	06 June 2002	WO 0243652 A2	06 Jun 2002
		AU 1846702 A	11 Jun 2002
		AU 2002218467 A2	11 Jun 2002
		AU 2002218467 B2	13 Jul 2006
		CA 2430296 A1	06 Jun 2002
		CN 1501797 A	02 Jun 2004
		EP 1347752 A2	01 Oct 2003
		JP 2004538245 A	24 Dec 2004
		KR 20030069176 A	25 Aug 2003
		US 2004029860 A1	12 Feb 2004
		US 2005013853 A1	20 Jan 2005
US 2004/0054014 A1	18 March 2004	US 2004054014 A1	18 Mar 2004
		CA 2460022 A1	20 Mar 2003
		EP 1425002 A1	09 Jun 2004
		JP 2005505559 A	24 Feb 2005
		KR 20050026903 A	16 Mar 2005
		US 2003087964 A1	08 May 2003
		US 6630454 B2	07 Oct 2003
		US 2004266882 A1	30 Dec 2004
		WO 03022258 A1	20 Mar 2003
US 6974837 B2	13 December 2005	US 2003096792 A1	22 May 2003
		US 6974837 B2	13 Dec 2005
		AU 5781799 A	14 Mar 2000
		AU 772303 B2	22 Apr 2004
		AU 2779301 A	24 Jul 2001
		AU 782567 B2	11 Aug 2005
		AU 8906201 A	26 Mar 2002
		AU 2001289062 B2	29 Mar 2007
		AU 2002245302 A1	12 Aug 2002
		AU 2004200875 A1	01 Apr 2004
		AU 2004200875 B2	26 Oct 2006

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2021/032715**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		AU 2005232311 A1	08 Dec 2005
		BR 9913325 A	02 Oct 2001
		CA 2341441 A1	02 Mar 2000
		CA 2396950 A1	19 Jul 2001
		CA 2422246 A1	21 Mar 2002
		CN 1735407 A	15 Feb 2006
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		EP 1107746 B1	13 Oct 2004
		EP 1246789 A1	09 Oct 2002
		EP 1246789 B1	31 May 2006
		EP 1320360 A1	25 Jun 2003
		EP 1475086 A2	10 Nov 2004
		EP 1632471 A1	08 Mar 2006
		HK 1088238 A1	03 Nov 2006
		HU 0103408 A2	29 May 2002
		ID 28638 A	21 Jun 2001
		IL 141531 A	31 May 2010
		JP 2002523366 A	30 Jul 2002
		JP 2003519675 A	24 Jun 2003
		JP 2004529850 A	30 Sep 2004
		KR 20060081725 A	13 Jul 2006
		KR 100887008 B1	04 Mar 2009
		KR 20010085553 A	07 Sep 2001
		KR 20080011354 A	01 Feb 2008
		NZ 510193 A	26 Sep 2003
		PL 346843 A1	25 Feb 2002
		PL 200264 B1	31 Dec 2008
		RU 2004116282 A	10 Nov 2005
		US 6331571 B1	18 Dec 2001
		US 6339106 B1	15 Jan 2002
		US 6375352 B1	23 Apr 2002
		US 6399826 B1	04 Jun 2002
		US 2002010198 A1	24 Jan 2002
		US 6476078 B2	05 Nov 2002
		US 2002188029 A1	12 Dec 2002
		US 6538034 B2	25 Mar 2003

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2021/032715**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 2002183281 A1	05 Dec 2002
		US 6710087 B2	23 Mar 2004
		US 2004180857 A1	16 Sep 2004
		US 6974838 B2	13 Dec 2005
		US 2003195261 A1	16 Oct 2003
		US 7071234 B2	04 Jul 2006
		US 2004067957 A1	08 Apr 2004
		US 2004092481 A1	13 May 2004
		US 2004116534 A1	17 Jun 2004
		US 2004162355 A1	19 Aug 2004
		WO 0010551 A2	02 Mar 2000
		WO 0151453 A1	19 Jul 2001
		WO 0222114 A2	21 Mar 2002
		WO 02060424 A2	08 Aug 2002
		ZA 200101498 B	22 Feb 2002

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2021/032715**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2002/060427 A2	08 August 2002	WO 02060427 A2	08 Aug 2002
		US 2002006964 A1	17 Jan 2002
		US 2003078303 A1	24 Apr 2003
WO 2002/060428 A2	08 August 2002	WO 02060428 A2	08 Aug 2002
		AU 4529897 A	05 Feb 1998
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		AU 4542893 A	24 Jan 1994
		CA 2138998 A1	06 Jan 1994
		EP 0647134 A1	12 Apr 1995
		JP H08500093 A	09 Jan 1996
		US 2002006963 A1	17 Jan 2002
		WO 9400114 A1	06 Jan 1994
WO 2020/043716 A1	05 March 2020	WO 2020043716 A1	05 Mar 2020
WO 2012/129352 A1	27 September 2012	WO 2012129352 A1	27 Sep 2012
		AU 2012203968 A1	11 Oct 2012
		CN 103547683 A	29 Jan 2014
		EP 2689030 A1	29 Jan 2014
		US 2014065615 A1	06 Mar 2014

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.