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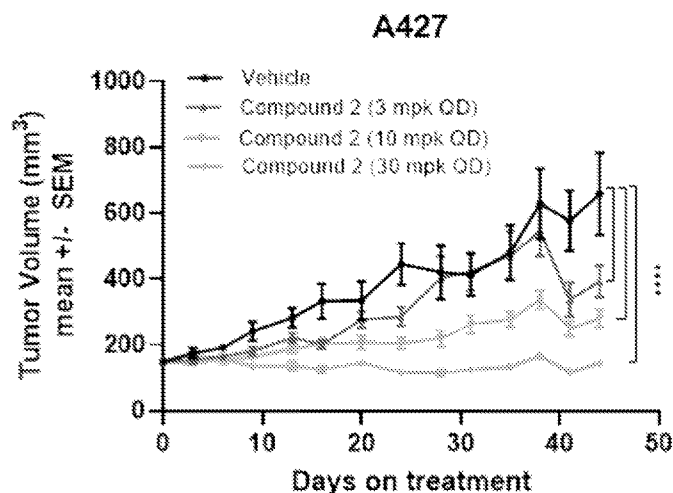


FIG. 10

(57) Abstract: The present disclosure describes methods of treating cancer in a subject having a splicing factor mutation comprising administering to the subject in need thereof a therapeutically effective amount of a S-adenosylmethionine (SAM)-competitive Protein Arginine Methyltransferase 5 (PRMT5) inhibitor.

TREATMENT OF CANCER USING A PRMT5 INHIBITOR

REFERENCE TO SEQUENCE LISTING

This application is being filed electronically via EFS-Web and includes an electronically submitted sequence listing in .txt format. The .txt file contains a sequence listing entitled
5 "PC72700_SEQListing_ST25.txt" created on December 13, 2021 and having a size of 1 KB. The sequence listing contained in this .txt file is part of the specification and is incorporated herein by reference in its entirety.

FIELD

The present disclosure relates to methods of treating cancer in a subject having a splicing
10 factor mutation comprising administering to the subject in need thereof a therapeutically effective amount of S-adenosylmethionine (SAM)-competitive Protein Arginine Methyltransferase 5 (PRMT5) inhibitor.

BACKGROUND

Protein Arginine Methyltransferase 5 (PRMT5) is the major type II arginine
15 methyltransferase that utilizes S-adenosylmethionine (SAM) to catalyze both mono- and symmetric di-methylation on protein substrates involved in a variety of cellular processes including transcription, cell signaling, mRNA translation, DNA repair, receptor trafficking, protein stability, and pre-mRNA splicing (see, e.g., Stopa, N. *et al.*, Cell Mol Life Sci, 72(11): 2041-59 (2015)). PRMT5 is a member of a hetero-octomeric protein complex, primarily localized in the
20 cytoplasm, composed of four PRMT5 proteins and four MEP50 proteins. Through additional interactions with accessory subunits, PRMT5 forms a larger methylosome complex, possessing symmetric di-methylation activity for proteins possessing RGG/RG motifs (see Branscombe, T.L., *et al.*, J Biol Chem, 276(35): 32971-6 (2001)). Cellular proteins targeted by PRMT5 methylation are particularly enriched in pre-mRNA regulation including splicing. Spliceosome
25 proteins SmD1, SmD3 and SmB/B' are methylated by PRMT5 in a co-translational manner, increasing their affinity for the tudor domain of the SMN1 protein, and facilitating assembly of small nuclear ribonucleoprotein (snRNP) complexes responsible for proper splice site recognition (see, e.g., Gonsalvez, G.B., *et al.*, J Cell Biol, 178(5): 733-40 (2007), Neuenkirchen, N., *et al.*, FEBS Lett, 582(14): p. 1997-2003 (2008)). PRMT5 genetic inhibition leads to
30 increased intron retention and exon skipping in pre-mRNAs, resulting in mRNA nonsense-mediated decay or alternatively spliced mRNAs. Proteins involved in pre-mRNA processing are highly enriched as cellular substrates of PRMT5 methylation.

PRMT5 over-expression has been observed in multiple cancers, including NSCLC (Non-Small Cell Lung Cancer), where enzyme function has been linked to enhanced tumor cell growth and poor patient survival (see, *e.g.*, Gu, Z., *et al.*, *Biochem J*, 446(2): 235-41 (2012), Lattouf, H., *et al.*, *Oncotarget*, 10(34): 3151-3153 (2019)). Cancer cells are dependent on PRMT5 enzyme activity for growth, as genetic inhibition or catalytic inhibition of PRMT5 blocks cancer cell proliferation and promotes apoptosis (See Gu (2012), *supra*). PRMT5 inhibitors have recently entered the clinic, such as GSK3326595 and JNJ-64619178, targeting multiple hematopoietic and solid tumor indications.

Specific splicing events induced by PRMT5 inhibition have particular relevance for the therapeutic targeting of PRMT5 in cancer. In p53 wild-type lymphoma models, PRMT5 inhibition leads to exon 6 skipping in MDM4, targeting the transcript for nonsense mediated decay, decreasing MDM4 protein levels, and subsequent activation of the p53 pathway. Activation of the p53-p21 pathway was linked to the anti-proliferative activity of PRMT5 inhibitors and TP53 mutations status correlated with response to PRMT5 inhibitors (see, Gerhart, S.V., *et al.*, *Sci Rep*, 8(1): 9711 (2018)). In lymphoma, activation of gene transcription driven through MYC over-expression generates higher reliance on proper splicing fidelity (see, Koh, C.M., *et al.*, *Nature*, 523(7558): 96-100 (2015)). Compensatory MYC-driven upregulation of snRNPs and PRMT5 create increased dependence on PRMT5 activity which can be therapeutically exploited in Eu-MYC driven mouse lymphoma models.

Recently, the Cancer Genome Atlas (TCGA) identified recurrent somatic mutations and copy number alterations in 119 splicing factor genes with known roles in RNA splicing, including snRNP assembly and branch point recognition (See, *e.g.*, Seiler, M., *et al.*, *Cell Rep*, 23(1): 282-296 e4 (2018)). These results highlight that perturbations in mRNA splicing represent a common feature of tumorigenesis. PRMT5 methylation of splice factors perturbed in cancer such as SRSF1 (See, Radzishchanskaya, A., *et al.*, *Nat Struct Mol Biol*, 26(11): 999-1012 (2019)) raises the possibility of targeting PRMT5 in cancers enriched in certain splice factors mutations. More recently, Fong *et al.* (Fong, J.Y., *et al.*, *Cancer Cell*, 36(2): 194-209 e9 (2019)) found PRMT5 inhibition reduces splicing fidelity, resulting in preferential killing of splice factor-mutant leukemias. Accordingly, development of or existence of mutations (*e.g.*, a splice factor mutation) is expected to arise from patients receiving cancer treatment with a PRMT5 inhibitor, and there is a need to identify and develop a potent PRMT5 inhibitor targeting cancers in patients with such mutations.

SUMMARY

In some aspects, embodiments herein relate to methods of treating cancer in a subject comprising administering to the subject in need thereof a therapeutically effective amount of a S-adenosylmethionine (SAM)-competitive Protein Arginine Methyltransferase 5 (PRMT5) inhibitor, wherein the subject has a splicing factor mutation.

In some aspects, embodiments herein relate to uses of a compound in the manufacture of a medicament for the treatment of a subject having a cancer, wherein the subject has a splicing factor mutation, and wherein the compound is a SAM-competitive PTMT5 inhibitor.

5 In some aspects, embodiments herein relate to medicaments comprising a therapeutically effective amount of a SAM-competitive PRMT5 inhibitor for use in treating cancer in a subject having a splicing factor mutation.

10 In some embodiments, there is provided a method of treating cancer in a subject comprising administering to the subject in need thereof a therapeutically effective amount of Compound 1 or a pharmaceutically acceptable salt thereof, or Compound 2 or a pharmaceutically acceptable salt thereof, wherein the subject has a splicing factor mutation.

In some embodiments, there is provided a SAM-competitive PRMT5 inhibitor for use in a method of treating cancer in a subject, wherein the subject has a splicing factor mutation.

15 In some embodiments, there is provided Compound 1 or a pharmaceutically acceptable salt thereof, or Compound 2 or a pharmaceutically acceptable salt thereof, for use in a method of treating cancer in a subject, wherein the subject has a splicing factor mutation.

In some embodiments, there is provided a use of a compound in the manufacture of a medicament for the treatment of a subject having a cancer, wherein the subject has a splicing factor mutation, and wherein the compound is Compound 1 or a pharmaceutically acceptable salt thereof, or Compound 2 or a pharmaceutically acceptable salt thereof.

20 In some embodiments, there is provided a medicament comprising a therapeutically effective amount of Compound 1 or a pharmaceutically acceptable salt thereof, or Compound 2, or a pharmaceutically acceptable salt thereof, for use in treating cancer in a subject having a splicing factor mutation.

25 In some embodiments, the SAM-competitive PRMT5 inhibitor is selected from the group consisting of Compound 1, Compound 2, LLY-283, and JNJ64619178.

In some embodiments, the SAM-competitive PRMT5 inhibitor is selected from the group consisting of Compound 1 and Compound 2.

30 In some embodiments, the subject has an existing splicing factor mutation or has developed a splicing factor mutation during the course of a prior cancer treatment, for example, a prior peptide-competitive PRMT5 inhibitor treatment.

In some embodiments, the subject is resistant or develops resistance to a prior PRMT5 inhibitor treatment.

In some embodiments, the splicing factor mutation is in a gene selected from the group consisting of RBM10, U2AF1, SF3B1, SRSF2, SF3A1, U2AF2, ELAVL2, FUBP1, and AQR.

35 In some embodiments, the splicing factor mutation is a loss of function mutation, for example, as a result of loss of protein expression.

In some embodiments, the splicing factor mutation is in RBM10.

In some embodiments, the splicing factor mutation in RBM10 is selected from the group consisting of G840fs, I348N, E705K, A695fs, P45L, I696fs, A552V, R171Q, G218C, Q481L, T694I, W723C, S846L, G935C, G961V, and H609fs.

In some embodiments, the splicing factor mutation in U2AF1 is S34F.

5 In some embodiments, the methods and uses described herein further comprise administering a therapeutically effective amount of a second therapeutic agent.

In some embodiments, the cancer for the treatment methods and uses described herein is selected from the group consisting of lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, 10 rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the 15 urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain stem glioma, and pituitary adenoma.

In some embodiments, the cancer is non-small cell lung cancer (NSCLC), lung 20 adenocarcinoma, head and neck squamous cell carcinoma (HNSCC), esophageal cancer, endometrial cancer, cervical cancer, bladder cancer, urothelial carcinoma, or pancreatic cancer.

In some embodiments, the cancer is relapsed, refractory, or metastatic.

DETAILED DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts chemical structure and co-crystal structures of PRMT5:MEP50 with adenosine, 25 Compound 1 and Compound 2.

FIG. 2 is the SPR (Surface Plasmon Resonance) graph of Compound 2; the table below shows binding kinetics of Compound 1 and Compound 2 with PRMT5:MEP50.

30 **FIG. 3** shows the SAM competition assay of Compound 2.

FIG. 4 depicts PRMT5:MEP50 co-crystal with Compound 2 with SAH overlay in the binding pocket.

35 **FIG. 5** shows the dose response of Compound 2 on cellular biomarker, symmetric di-methyl arginine in A427 cells at 72hr.

FIG. 6 depicts the proliferation curves (7 day Cell Titer Glo) of NSCLC cells treated with Compound 2.

FIG. 7 depicts the Compound 2 induction of cell cycle arrest in A427 and NCI-H1975 cells at Day 5 post-treatment.

FIG. 8 depicts Compound 2 showing dose dependent increases in apoptotic markers in A427 cells.

FIG. 9 depicts Compound 2 inducing senescence in A549 cells treated for 10 days.

FIG. 10 depicts tumor growth inhibition of A427 xenograft with Compound 2 treatment for 44 days.

FIG. 11 depicts SDMA modulation in A427 tumors taken at study endpoint.

FIG. 12 depicts body weight measurements of A427 xenograft throughout study duration.

FIG. 13 shows tumor growth inhibition of NCI-H441 xenograft with Compound 2 treatment for 36 days.

FIG. 14 depicts SDMA modulation in NCI-H441 tumors taken at study endpoint.

FIG. 15 depicts body weight of NCI-H441 xenograft throughout study duration.

FIG. 16 shows tumor growth inhibition of NSCLC PDX NSX-26183 xenograft, an RBM10 copy number loss model, with Compound 2 treatment for 29 days.

FIG. 17 shows tumor growth inhibition of NSCLC PDX NSX-26130 xenograft, an RBM10 copy number loss model, with Compound 2 treatment for 28 days.

FIG. 18 shows tumor growth inhibition of NSCLC PDX NSX-26109 xenograft, an RBM10 copy number loss model, with Compound 2 treatment for 25 days.

FIG. 19 depicts growth inhibitory concentrations (GI50) of NSCLC cells in a 7 proliferation assay with Compound 2.

FIG. 20 depicts pathway enrichment for genes whose expression associated with sensitivity to PRMT5 inhibition in NSCLC.

FIG. 21 depicts alternative splicing events that stratify sensitive and resistant NSCLC cell lines to Compound 2.

FIG. 22 depicts RBM10 mutant vs. WT NSCLC cell line sensitivity to Compound 1 treatment and RBM10 protein expression in NSCLC cell lines.

FIG. 23 depicts isogenic NCI-H1975 cells +/- RBM10 expression show differentiated response to Compound 2 treatment.

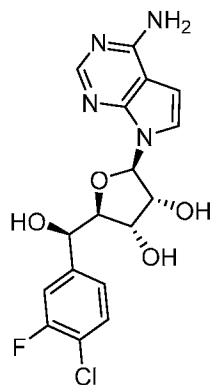
DETAILED DESCRIPTION

All patents, applications, published applications and other publications are incorporated by reference in their entirety. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by those ordinary skill in the art to which the disclosure belongs.

The following definitions are provided to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure. Heading used herein are for organizational purposes only and in no way limit the embodiments described herein.

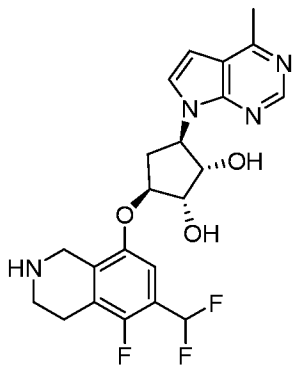
Definitions

As used herein, "Compound 1" refers to (2S,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((R)-(4-chloro-3-fluorophenyl)(hydroxy)methyl)tetrahydrofuran-3,4-diol having the structure of:



which is described in U.S. Patent No. 10,428,104, and the content is incorporated by reference in its entirety.

As used herein, "Compound 2" refers to (1S,2S,3S,5R)-3-((6-(difluoromethyl)-5-fluoro-1,2,3,4-tetrahydroisoquinolin-8-yl)oxy)-5-(4-methyl-7H-pyrrolo[2,3-d]pyrimidin-7-yl)cyclopentane-1,2-diol having the structure of:



5 which is described in U.S. Patent No. 10,220,037, and the content is incorporated by reference in its entirety.

As used herein, the singular form "a", "an", and "the" include plural references unless indicated otherwise. For example, "a" substituent includes one or more substituents.

10 The term "patient" or "subject" refer to any single subject for which therapy is desired or that is participating in a clinical trial, epidemiological study or used as a control, including humans and mammalian veterinary patients such as cattle, horses, dogs and cats. In certain preferred embodiments, the subject is a human. In some embodiments, the subject is a human having a splicing factor mutation described herein.

15 The term "treat" or "treating" cancer or an associated condition or disease as used herein means to administer a therapy according to the present invention to a subject having cancer or an associated condition or disease to achieve at least one positive therapeutic effect. The term "treatment", as used herein, unless otherwise indicated, refers to the act of treating as "treating" is defined immediately above. The term "treating" also includes adjuvant and neo-adjuvant treatment of a subject.

20 As used in herein, "administering" refers to the delivery of a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Exemplary routes of administration include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration
25 other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as in vivo electroporation. A therapeutic agent can be administered via a non-

parenteral route, or orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

5 The terms “treatment regimen”, “dosing protocol” and “dosing regimen” are used interchangeably to refer to the dose and timing of administration of each therapeutic agent in a combination of the invention.

 “Ameliorating” means a lessening or improvement of one or more symptoms upon treatment with a combination described herein, as compared to not administering the
10 combination. “Ameliorating” also includes shortening or reduction in duration of a symptom.

 As used herein, an “effective dosage”, “effective amount”, or “therapeutically effective amount” of a drug, compound or pharmaceutical composition is an amount sufficient to effect any one or more beneficial or desired, including biochemical, histological and/or behavioural symptoms, of the disease, its complications and intermediate pathological phenotypes presenting
15 during development of the disease. For therapeutic use, a “therapeutically effective amount” refers to that amount of a compound being administered which will relieve to some extent one or more of the symptoms of the disorder being treated. An effective dosage can be administered in one or more administrations. For the purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or
20 therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of drug, compound or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound or pharmaceutical composition.

 As used herein, the term “pharmaceutically acceptable salt(s)” refers to a salt prepared from a pharmaceutically acceptable non-toxic acid or base including an inorganic acid and base
25 and an organic acid and base. Suitable pharmaceutically acceptable salt include, but are not limited to acetate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate/sulfate, borate, camsylate, citrate, edisylate, esylate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, isethionate, lactate, malate, maleate, malonate, mesylate, methylsulfate,
30 naphthylate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, saccharate, stearate, succinate, tartrate, tosylate and trifluoroacetate, aluminium, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine, tosylate, and zinc salts. For a review on suitable salts, see “Handbook of
35 Pharmaceutical Salts: Properties, Selection, and Use” by Stahl and Wermuth (Wiley-VCH, Weinheim, Germany, 2002), the disclosure of which is incorporated herein by reference in its entirety.

As used herein, the term "pharmaceutically acceptable" refers to those properties and/or substances which are acceptable to the patient from a pharmacological/toxicological point of view and to the manufacturing pharmaceutical chemist from a physical/chemical point of view regarding composition, formulation, stability, patient acceptance and bioavailability.

5 As used herein, S-adenosylmethionine (SAM)-competitive Protein Arginine Methyltransferase 5 (PRMT) inhibitor means a PRMT5 inhibitor that competes with SAM for binding to PRMT5. Examples of a SAM-competitive PRMT5 inhibitor include, but are not limited to, Compound 1, Compound 2, LLY-283, and JNJ64619178.

As used herein, a peptide-competitive PRMT5 inhibitor means a PRMT5 inhibitor that
10 competes with the peptide substrate for binding to PRMT5. Examples of a peptide-competitive PRMT5 inhibitor include, but are not limited to, EPZ015666.

"Chemotherapeutic agent" is a chemical compound useful in the treatment of cancer and/or cancer-associated disease. Classes of chemotherapeutic agents include, but are not limited to: alkylating agents, antimetabolites, kinase inhibitors, spindle poison plant alkaloids, cytotoxic/antitumor antibiotics, topoisomerase inhibitors, photosensitizers, anti-estrogens and
15 selective estrogen receptor modulators (SERMs), anti-progesterones, estrogen receptor down-regulators (ERDs), estrogen receptor antagonists, leutinizing hormone-releasing hormone agonists, anti-androgens, aromatase inhibitors, EGFR inhibitors, VEGF inhibitors, and anti-sense oligonucleotides that inhibit expression of genes implicated in abnormal cell proliferation or tumor
20 growth. Chemotherapeutic agents are further described herein.

"Chemotherapy" as used herein, refers to a chemotherapeutic agent, as defined above, or a combination of two, three or four chemotherapeutic agents, for the treatment of cancer and/or cancer-associated disease. When chemotherapy consists more than one chemotherapeutic agent, the chemotherapeutic agents can be administered to the patient on the same day or on
25 different days in the same treatment cycle.

As used herein, the term "refractory" or "resistant" refers to a circumstance where patients, even after intensive treatment, have residual cancer cells in their body.

The present disclosure may be understood more readily by reference to the following detailed description of the embodiments of the invention and the Examples included herein. It is
30 to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting. It is further to be understood that unless specifically defined herein, the terminology used herein is to be given its traditional meaning as known in the relevant art.

The disclosure described herein may be suitably practiced in the absence of any
35 element(s) not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms.

Methods, Uses, and Medicaments

The treatment methods and uses of the present disclosure are directed to treating cancer in a subject comprising administering to the subject in need thereof a therapeutically effective amount of a SAM-competitive PRMT5 inhibitor, wherein the subject has a splicing factor mutation. In some embodiments, the SAM-competitive PRMT5 inhibitor is selected from the group consisting of Compound 1, Compound 2, LLY-283, and JNJ64619178. In some embodiments, the SAM-competitive PRMT5 inhibitor is Compound 1 or Compound 2.

In some embodiments, provided herein is a method of treating cancer in a subject comprising administering to the subject in need thereof a therapeutically effective amount of Compound 2, or a pharmaceutically acceptable salt thereof, wherein the subject has a splicing factor mutation.

In some embodiments, provided herein is a use of Compound 2 or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of a subject having a cancer, wherein the subject has a splicing factor mutation.

In some embodiments, provided herein is a medicament comprising a therapeutically effective amount of Compound 2 or a pharmaceutically acceptable salt thereof, for use in treating cancer in a subject having a splicing factor mutation.

In some embodiments, the subject has an existing splicing factor mutation or has developed a splicing factor mutation during the course of a prior cancer treatment (e.g., a prior peptide-competitive PRMT5 inhibitor treatment).

In some embodiments, the subject is resistant to a prior PRMT5 inhibitor treatment.

In some embodiments, the subject is suffering from cancer or has been diagnosed with cancer having a splicing factor mutation. In some embodiments, the subject is suffering from cancer that is positive for one or more mutations in a spliceosome gene or protein. In some embodiments, the subject is suffering from cancer having RBM10 mutations. In some embodiments, the subject is suffering from lung cancer.

In some embodiments, the splicing factor mutation is in a gene selected from the group consisting of RNA-binding motif 10 (RBM10), U2AF1, SF3B1, SRSF2, SF3A1, U2AF2, ELAVL2, FUBP1, and AQR.

In some embodiments, the splicing factor mutation is a loss of function mutation (such as loss of protein expression). The type of mutation causing the loss of function or loss of protein expression of any of these splicing factor mutations described herein includes, but is not limited to, frameshift mutation, missense mutation, deleterious mutation, non-sense mutation, and premature stop codons in the gene sequence.

In some embodiments, the splicing factor mutation in RBM10 is selected from the group consisting of G840fs, I348N, E705K, A695fs, P45L, I696fs, A552V, R171Q, G218C, Q481L, T694I, W723C, S846L, G935C, G961V, and H609fs.

In some embodiments, the splicing factor mutation in U2AF1 is S34F.

In one aspect, provided herein is a method of treating cancer in a subject comprising administering to the subject in need thereof a therapeutically effective amount of Compound 2, or a pharmaceutically acceptable salt thereof, wherein the subject has a splicing factor mutation
5 in RBM10.

In another aspect, provided is a use of Compound 2 or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of a subject having a cancer, wherein the subject has a splicing factor mutation in RBM10Compound 2

In another aspect, provided herein is a medicament comprising a therapeutically effective
10 amount of Compound 2 or a pharmaceutically acceptable salt thereof, for use in treating cancer in a subject having a splicing factor mutation in RBM10.

The types of cancer as described in the treatment methods and uses as disclosed herein include, but are not limited to, lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal
15 cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the
20 urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain stem glioma, and pituitary adenoma.

In some embodiments, indications treated by the methods and uses disclosed herein
25 include, but are not limited to, non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), esophageal cancer, endometrial cancer, cervical cancer, bladder cancer, urothelial carcinoma, and pancreatic cancer.

In another aspect, provided herein is a method of treating cancer in a subject comprising administering to the subject in need thereof a therapeutically effective amount of (SAM)-
30 competitive PRMT5 inhibitor, wherein the subject has a splicing factor mutation, and further comprising administering an effective amount of a second therapeutic agent.

Combination Therapies

In some aspects, provided herein is a method of treating cancer in a subject comprising
35 administering to the subject in need thereof a therapeutically effective amount of Compound 2, or a pharmaceutically acceptable salt thereof, wherein the subject has a splicing factor mutation, and further comprising administering an effective amount of a second therapeutic agent.

In some embodiments, the second therapeutic agent may comprise one or more of a chemotherapeutic agents, a biotherapeutic agent, an immunomodulating agent, a proteasome inhibitor, and a corticosteroid. Further therapeutic agents for use in the combination therapy of the present invention include a cancer vaccine, immune cell therapy (e.g., CAR-T cell-based therapy), radiotherapy, a vaccine, a cytokine therapy (e.g., immunostimulatory cytokines including various signaling proteins that stimulate immune response, such as interferons, interleukins, and hematopoietic growth factors), a targeted cytokine, an inhibitor of other immunosuppressive pathways, an inhibitors of angiogenesis, a T cell activator, an inhibitor of a metabolic pathway, an mTOR (mechanistic target of rapamycin) inhibitor (e.g., rapamycin, rapamycin derivatives, sirolimus, temsirolimus, everolimus, and deforolimus), an inhibitor of an adenosine pathway, a gamma secretase inhibitor (e.g., nirogacestat), a tyrosine kinase inhibitor including but not limited to INLYTA®, ALK (anaplastic lymphoma kinase) inhibitors (e.g., crizotinib, ceritinib, alectinib, and sunitinib), a BRAF inhibitor (e.g., vemurafenib and dabrafenib), a PI3K inhibitor, a HPK1 inhibitor, an epigenetic modifier, an inhibitors or depletor of Treg cells and/or of myeloid-derived suppressor cells, a JAK (Janus Kinase) inhibitor (e.g., ruxolitinib and tofacitinib, varicitinib, filgotinib, gandotinib, lestaurtinib, momelotinib, pacritinib, and upadacitinib), a STAT (Signal Transducers and Activators of Transcription) inhibitor (e.g., STAT1, STAT3, and STAT5 inhibitors such as fludarabine), a cyclin-dependent kinase (CDK) or other cell cycle inhibitor, an immunogenic agent (for example, attenuated cancerous cells, tumor antigens, antigen presenting cells such as dendritic cells pulsed with tumor derived antigen or nucleic acids, a MEK inhibitor (e.g., trametinib, cobimetinib, binimetinib, and selumetinib), a GLS1 inhibitor, a PARP inhibitor (e.g., talazoparib, olaparib, rucaparib, niraparib), an oncolytic virus, gene therapies including DNA, RNA delivered directly or by adeno-associated viruses (AAV) or nanoparticles, an innate immune response modulator (e.g., TLRs, KIR, NKG2A), an IDO (Indoleamine-pyrrole 2,3-dioxygenase) inhibitor, a PRR (Pattern Recognition Receptors) agonist, and cells transfected with genes encoding immune stimulating cytokines such as but not limited to GM-CSF).

The treatment methods and uses described herein may comprise administering of one or more chemotherapeutic agents. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan,

novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, especially calicheamicin gamma1I and calicheamicin phil1, see, e.g., Agnew, Chem. Intl. Ed. Engl., 33:183-186 (1994); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomorphores), aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, carabycin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanol, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; FOLFOX including folinic acid, 5-FU and oxaliplatin; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., paclitaxel and docetaxel; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as carboplatin; cisplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

The treatment methods and uses described herein may comprise administering one or more biotherapeutic agents such as antibodies. Examples of antibodies including, but are not limited to, an anti-CTLA-4 antibody, an anti-CD3 antibody, an anti-CD4 antibody, an anti-CD8 antibody, an anti-4-1BB antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-TIM3

antibody, an anti-LAG3 antibody, an anti-TIGIT antibody, an anti-OX40 antibody, an anti-IL-7Ralpha (CD127) antibody, an anti-IL-8 antibody, an anti-IL-15 antibody, an anti-HVEM antibody, an anti-BTLA antibody, an anti-CD38 antibody, an anti-CD40 antibody, an anti-CD40L antibody, anti-CD47 antibody, an anti-CSF1R antibody, an anti-CSF1 antibody, an anti-IL-7R antibody, an
 5 anti-MARCO antibody, an anti-CXCR4 antibodies, an anti-VEGF antibody, an anti-VEGFR1 antibody, an anti-VEGFR2 antibody, an anti-TNFR1 antibody, an anti-TNFR2 antibody, an anti-CD3 bispecific antibody, an anti-CD19 antibody, an anti-CD20, an anti-Her2 antibody, an anti-EGFR antibody, an anti-ICOS antibody, an anti-CD22 antibody, an anti-CD52 antibody, an anti-CCR4 antibody, an anti-CCR8 antibody, an anti-CD200R antibody, an anti-VISG4 antibody, an
 10 anti-CCR2 antibody, an anti-LILRB2 antibody, an anti-CXCR4 antibody, an anti-CD206 antibody, an anti-CD163 antibody, an anti-KLRG1 antibody, an anti-FLT3 antibody, an anti-B7-H4 antibody, an anti-B7-H3 antibody, an KLRG1 antibody, a BTN1A1 antibody, a BCMA antibody, an anti-SLAMF7 antibody, an anti-avb8 antibody, an anti-CD80 antibody, or an anti-GITR antibody.

In some embodiments, the second therapeutic agent is an anti-PD-1 antibody or an anti-
 15 PD-L1 antibody. Examples of anti-PD-1 and anti-PD-L1 antibodies include, but are not limited to, atezolizumab (TECENTRIQ®, MPDL3280A, Roche Holding AG), durvalumab (IMFINZI®, AstraZeneca PLC), nivolumab (OPDIVO®, ONO-4538, BMS-936558, MDX1106, Bristol-Myers Squibb Company), pembrolizumab (KEYTRUDA®, MK-3475, lambrolizumab, Merck & Co., Inc.), BCD-100 (BIOCAD Biopharmaceutical Company), tislelizumab (BGB-A317, BeiGene
 20 Ltd./Celgene Corporation), genolimzumab (CBT-501, CBT Pharmaceuticals), CBT-502 (CBT Pharmaceuticals), GLS-010 (Harbin Gloria Pharmaceuticals Co., Ltd.), sintilimab (IBI308, Innovent Biologics, Inc.), WBP3155 (CStone Pharmaceuticals Co., Ltd.), AMP-224 (GlaxoSmithKline plc), BI 754091 (Boehringer Ingelheim GmbH), BMS-936559 (Bristol-Myers Squibb Company), CA-170 (Aurigene Discovery Technologies), FAZ053 (Novartis AG),
 25 spartalizumab (PDR001, Novartis AG), LY3300054 (Eli Lilly & Company), MEDI0680 (AstraZeneca PLC), PDR001 (Novartis AG), sasanlimab (PF-06801591, Pfizer Inc.), cemiplimab (LIBTAYO®, REGN2810, Regeneron Pharmaceuticals, Inc.), camrelizumab (SHR-1210, Incyte Corporation), TSR-042 (Tesaro, Inc.), AGEN2034 (Agenus Inc.), CX-072 (CytomX Therapeutics, Inc.), JNJ-63723283 (Johnson & Johnson), MGD013 (MacroGenics, Inc.), AN-2005 (Adlai
 30 Nortye), ANA011 (AnaptysBio, Inc.), ANB011 (AnaptysBio, Inc.), AUNP-12 (Pierre Fabre Medicament S.A.), BBI-801 (Sumitomo Dainippon Pharma Co., Ltd.), BION-004 (Aduro Biotech), CA-327 (Aurigene Discovery Technologies), CK-301 (Fortress Biotech, Inc.), ENUM 244C8 (Enumeral Biomedical Holdings, Inc.), FPT155 (Five Prime Therapeutics, Inc.), FS118 (F-star Alpha Ltd.), hAb21 (Stainwei Biotech, Inc.), J43 (Transgene S.A.), JTX-4014 (Jounce
 35 Therapeutics, Inc.), KD033 (Kadmon Holdings, Inc.), KY-1003 (Kymab Ltd.), MCLA-134 (Merus B.V.), MCLA-145 (Merus B.V.), PRS-332 (Pieris AG), SHR-1316 (Atridia Pty Ltd.), STI-A1010 (Sorrento Therapeutics, Inc.), STI-A1014 (Sorrento Therapeutics, Inc.), STI-A1110 (Les Laboratoires Servier), and XmAb20717 (Xencor, Inc.).

The second therapeutic agent for use in the methods and uses described in the present invention may be directed or targeted to, for example, 5T4; A33; alpha-folate receptor 1 (e.g., mirvetuximab soravtansine); Alk-1; BCMA (e.g. see WO2016166629 and others disclosed herein); BTN1A1 (e.g., see WO2018222689); CA19-9; CA-125 (e.g., abagovomab);

5 Carboanhydrase IX; CCR2; CCR4 (e.g. mogamulizumab); CCR5 (e.g. leronlimab); CCR8; CD3 [e.g. blinatumomab (CD3/CD19 bispecific), PF-06671008 (CD3/P-cadherin bispecific), PF-06863135 (CD3/BCMA bispecific)]; CD19 (e.g., blinatumomab, MOR208); CD20 (e.g., ibritumomab tiuxetan, obinutuzumab, ofatumumab, rituximab, ublituximab); CD22 (inotuzumab ozogamicin, moxetumomab pasudotox); CD25; CD28; CD30 (e.g., brentuximab vedotin); CD33

10 (e.g., gemtuzumab ozogamicin); CD38 (e.g., daratumumab, daratumumab and hyaluronidase, and isatuximab), CD40; CD-40L; CD44v6; CD47 (e.g., Hu5F9-G4, CC-90002, SRF231, B6H12); CD52 (e.g., alemtuzumab); CD56; CD63; CD79 (e.g. polatuzumab vedotin); CD80; CD86; CD123; CD276 / B7-H3 (e.g., omburtamab); CDH17; CEA; ClhCG; CTLA-4 (e.g., ipilimumab, tremelimumab), CXCR4; desmoglein 4; DLL3 (e.g., rovalpituzumab tesirine); DLL4; E-cadherin;

15 EDA; EDB; EFNA4; EGFR (e.g., cetuximab, depatuxizumab mafodotin, necitumumab, panitumumab); EGFRvIII; Endosialin; EpCAM (e.g., oportuzumab monatox); FAP; Fetal Acetylcholine Receptor; FLT3 (e.g., see WO2018/220584); 4-1BB (CD137) [e.g., utomilumab/PF-05082566 (see WO2012/032433) or urelumab/BMS-663513], GD2 (e.g., dinutuximab, 3F8); GD3; G1TR (e.g., TRX518); GloboH; GM1; GM2; HER2/neu [e.g. margetuximab, pertuzumab,

20 trastuzumab; ado-trastuzumab emtansine, trastuzumab duocarmazine, PF-06804103 (see US8828401)]; HER3; HER4; ICOS; IL-10; ITG-AvB6; LAG-3 (e.g., relatlimab, IMP701); Lewis-Y; LG; Ly-6; M-CSF [e.g., PD-0360324 (see US7326414)]; (membrane-bound) IgE; MCSP; mesothelin; MIS Receptor type II; MUC1; MUC2; MUC3; MUC4; MUC5AC; MUC5B; MUC7; MUC16; Notch1; Notch3; Nectin-4 (e.g., enfortumab vedotin); OX40 [e.g. PF-04518600 (see

25 US7960515)]; P-Cadherin [e.g., PF-06671008 (see WO2016/001810)]; PCDHB2; PD-1 [e.g., BCD-100, camrelizumab, cemiplimab, genolimzumab (CBT-501), MEDI0680, nivolumab, pembrolizumab, sasanlimab (PF-06801591, see WO2016/092419), sintilimab, spartalizumab, STI-A1110, tislelizumab, TSR-042, and others disclosed herein]; PD-L1 (e.g., atezolizumab, durvalumab, BMS-936559 (MDX-1105), LY3300054, and others disclosed herein); PDGFRA

30 (e.g., olaratumab); Plasma Cell Antigen; PolySA; PSCA; PSMA; PTK7 [e.g., PF-06647020 (see US9409995)]; Ror1; SAS; SLAMF7 (e.g. elotuzumab); SHH; SIRPa (e.g., ED9, Effi-DEM); STEAP; sTn; TGF-beta; TIGIT; TIM-3; TMPRSS3; TNF-alpha precursor; TROP-2 (e.g., sacituzumab govitecan); TSPAN8; VEGF (e.g., bevacizumab, brolucizumab); VEGFR1 (e.g., ranibizumab); VEGFR2 (e.g., ramucirumab, ranibizumab); and Wue-1.

35

Treatment

Each therapeutic agent in the treatment methods and uses described herein can be administered either alone or in a medicament (also referred to herein as a pharmaceutical

composition) which comprises the therapeutic agent and one or more pharmaceutically acceptable carriers, excipients and diluents, according to standard pharmaceutical practice. In one embodiment, the therapeutic agent is Compound 1 or a pharmaceutically acceptable salt thereof. In one embodiment, the therapeutic agent is Compound 2 or a pharmaceutically acceptable salt thereof.

Each therapeutic agent in the methods described herein can be administered, either alone as a monotherapy, or in combination with one or more additional therapeutic agents, by any suitable enteral route or parenteral route of administration. The term "enteral route" of administration refers to the administration via any part of the gastrointestinal tract. Examples of enteral routes include oral, mucosal, buccal, and rectal route, or intragastric route. "Parenteral route" of administration refers to a route of administration other than enteral route. Examples of parenteral routes of administration include intravenous, intramuscular, intradermal, intraperitoneal, intratumor, intravesical, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, transtracheal, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal, subcutaneous, or topical administration. The therapeutic agents of the disclosure can be administered using any suitable method, such as by oral ingestion, nasogastric tube, gastrostomy tube, injection, infusion, implantable infusion pump, and osmotic pump. The suitable route and method of administration may vary depending on a number of factors such as the specific therapeutic agent being used, the rate of absorption desired, specific formulation or dosage form used, type or severity of the disorder being treated, the specific site of action, and conditions of the patient.

Oral administration of a solid dose form of a therapeutic agent may be, for example, presented in discrete units, such as hard or soft capsules, pills, cachets, lozenges, or tablets, each containing a predetermined amount of at least one therapeutic agent. In another aspect, the oral administration may be in a powder or granule form. In another aspect, the oral dose form is sub-lingual, such as, for example, a lozenge. In such solid dosage forms, therapeutic agents are ordinarily combined with one or more adjuvants. Such capsules or tablets may contain a controlled-release formulation. In the case of capsules, tablets, and pills, the dosage forms also may comprise buffering agents or may be prepared with enteric coatings.

In another aspect, oral administration of a therapeutic agent may be in a liquid dose form. Liquid dosage forms for oral administration include, for example, pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art (e.g., water). Such compositions also may comprise adjuvants, such as wetting, emulsifying, suspending, flavoring (e.g., sweetening), and/or perfuming agents.

Accordingly, for example, in some embodiments, Compound 1 or a pharmaceutically acceptable salt thereof, or Compound 2 or a pharmaceutically acceptable salt thereof, is administered as a monotherapy in a dosage form selected from one or more tablets, one or more

capsules, a liquid solution, a liquid suspension or a syrup. In some embodiments the dosage form is one or more tablets, preferably a single tablet.

In some aspects, therapeutic agents are administered in a parenteral dose form. "Parenteral administration" includes, for example, subcutaneous injections, intravenous
5 injections, intraperitoneal injections, intramuscular injections, intrasternal injections, and infusion. Injectable preparations (*i.e.*, sterile injectable aqueous or oleaginous suspensions) may be formulated according to the known art using suitable dispersing, wetting, and/or suspending agents, and include depot formulations.

For example, in some embodiments, Compound 1 or a pharmaceutically acceptable salt
10 thereof, or Compound 2 or a pharmaceutically acceptable salt thereof, is administered in a dosage form selected from one or more tablets, one or more capsules, a liquid solution, a liquid suspension or a syrup, and a second therapeutic agent (*e.g.*, a chemotherapeutic agent such as docetaxel or a biotherapeutic agent such as a PD-1 or PD-L1 antibody) is administered intravenously.

In some aspects, therapeutic agents are administered in a topical dose form. "Topical
15 administration" includes, for example, transdermal administration, such as via transdermal patches or iontophoresis devices, intraocular administration, or intranasal or inhalation administration. Compositions for topical administration also include, for example, topical gels, sprays, ointments, and creams. A topical formulation may include a compound that enhances
20 absorption or penetration of the active ingredient through the skin or other affected areas. When therapeutic agents are administered by a transdermal device, administration will be accomplished using a patch either of the reservoir and porous membrane type or of a solid matrix variety. Typical formulations for this purpose include gels, hydrogels, lotions, solutions, creams, ointments, dusting powders, dressings, foams, films, skin patches, wafers, implants, sponges,
25 fibers, bandages and microemulsions. Liposomes may also be used. Typical carriers include alcohol, water, mineral oil, liquid petrolatum, white petrolatum, glycerin, polyethylene glycol and propylene glycol. Penetration enhancers may be incorporated--see, for example, Finnin and Morgan, *J. Pharm. Sci.*, 88 (10), 955-958 (1999).

Other carrier materials and modes of administration known in the pharmaceutical art may
30 also be used with therapeutic agents. The above considerations in regard to effective formulations and administration procedures are well known in the art and are described in standard textbooks. Formulation of drugs is discussed in, for example, Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa., 1975; Liberman *et al.*, Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y., 1980; and Kibbe *et al.*,
35 Eds., *Handbook of Pharmaceutical Excipients* (3^{sup}.rd Ed.), American Pharmaceutical Association, Washington, 1999.

For the combination treatment, selecting a dosage regimen (also referred to herein as an administration regimen) for the methods described herein may depend on several factors,

including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells, tissue or organ in the subject being treated. Preferably, a dosage regimen maximizes the amount of each therapeutic agent delivered to the patient consistent with an acceptable level of side effects. Accordingly, the dose amount and dosing frequency of each therapeutic agent or chemotherapeutic agent in the combination depends in part on the particular therapeutic agent, the severity of the cancer being treated, and patient characteristics. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available. See, e.g., Wawrzynczak (1996) Antibody Therapy, Bios Scientific Pub. Ltd, Oxfordshire, UK; Kresina (ed.) (1991) Monoclonal Antibodies, Cytokines and Arthritis, Marcel Dekker, New York, NY; Bach (ed.) (1993) Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases, Marcel Dekker, New York, NY; Baert *et al.* (2003) New Engl. J. Med. 348:601-608; Milgrom *et al.* (1999) New Engl. J. Med. 341:1966-1973; Slamon *et al.* (2001) New Engl. J. Med. 344:783-792; Beniaminovitz *et al.* (2000) New Engl. J. Med. 342:613-619; Ghosh *et al.* (2003) New Engl. J. Med. 348:24-32; Lipsky *et al.* (2000) New Engl. J. Med. 343:1594-1602; Physicians' Desk Reference 2003 (Physicians' Desk Reference, 57th Ed); Medical Economics Company; ISBN: 1563634457; 57th edition (November 2002). Determination of the appropriate dosage regimen may be made by the clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment, and will depend, for example, the patient's clinical history (e.g., previous therapy), the type and stage of the cancer to be treated and biomarkers of response to one or more of the therapeutic agents in the combination therapy.

In some aspects, a subject can be administered with a fixed dose of each therapeutic agent (e.g., Compound 2 in monotherapy or in combination therapy with one or more additional therapeutic agents such as chemotherapeutic agents or biotherapeutic agents) of about or of at least about 0.05 µg, 0.2 µg, 0.5 µg, 1 µg, 10 µg, 100 µg, 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg, 45 mg, 46 mg, 47 mg, 48 mg, 49 mg, 50 mg, 51 mg, 52 mg, 53 mg, 54 mg, 55 mg, 56 mg, 57 mg, 58 mg, 59 mg, 60 mg, 61 mg, 62 mg, 63 mg, 64 mg, 65 mg, 66 mg, 67 mg, 68 mg, 69 mg, 70 mg, 75 mg, 80 mg, 90 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg, 225 mg, 250 mg, 275 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 350 mg, 700 mg, 750 mg, 800 mg, 900 mg, 1000 mg or 1500 mg, or higher. The fixed dose may be administered at intervals of, e.g., daily, every other day, three times per week, or one time each week, two weeks, three weeks, monthly, once every 2 months, once every 3 months, once every 4 months, etc.

In some aspects, each therapeutic agent (e.g., Compound 2 in monotherapy or in combination therapy with one or more additional therapeutic agents such as chemotherapeutic

agents or biotherapeutic agents) of the treatment methods described herein can be administered to a subject at a dose from about 0.05 $\mu\text{g/kg}$ to about 1000 mg/kg, from about 2 mg/kg to about 900 mg/kg, from about 3 mg/kg to about 800 mg/kg, from about 4 mg/kg to about 700 mg/kg, from about 5 mg/kg to about 600 mg/kg, from about 6 mg/kg to about 550 mg/kg, from about 7 mg/kg to about 500 mg/kg, from about 8 mg/kg to about 450 mg/kg, from about 9 mg/kg to about 400 mg/kg, from about 5 mg/kg to about 200 mg/kg, from about 2 mg/kg to about 150 mg/kg, from about 5 mg/kg to about 100 mg/kg, from about 10 mg/kg to about 100 mg/kg, or from about 10 mg/kg to about 60 mg/kg. For example, the second therapeutic agent can be administered to a subject at a dose of at least about 0.05 $\mu\text{g/kg}$, 0.2 $\mu\text{g/kg}$, 0.5 $\mu\text{g/kg}$, 1 $\mu\text{g/kg}$, 10 $\mu\text{g/kg}$, 100 $\mu\text{g/kg}$, 0.2 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 3.0 mg/kg, 5.0 mg/kg, 10 mg/kg, 25 mg/kg, 50 mg/kg body weight or more. See, e.g., Yang et al. (2003) New Engl. J. Med. 349:427-434; Herold et al. (2002) New Engl. J. Med. 346:1692-1698; Liu et al. (1999) J. Neurol. Neurosurg. Psych. 67:451-456; Portielji et al. (20003) Cancer Immunol. Immunother. 52:133-144.

In some aspects, each therapeutic agent (e.g., Compound 2 in monotherapy or in combination therapy with one or more additional therapeutic agents such as chemotherapeutic agents or biotherapeutic agents) of the treatment methods described herein can be administered to a subject at a dose from about 1 mg/m² to about 3000 mg/m², from about 2 mg/m² to about 2000 mg/m², from about 3 mg/m² to about 1000 mg/m², from about 4 mg/m² to about 750 mg/m², from about 5 mg/m² to about 600 mg/m², from about 6 mg/m² to about 550 mg/m², from about 7 mg/m² to about 500 mg/m², from about 8 mg/m² to about 450 mg/m², from about 9 mg/m² to about mg/m². For example, the second therapeutic agent can be administered to a subject at a dose of at least about 5 mg/m², 10 mg/m², 15 mg/m², 20 mg/m², 25 mg/m², 30 mg/m², 35 mg/m², 40 mg/m², 45 mg/m², 50 mg/m², 55 mg/m², 60 mg/m², 65 mg/m², 70 mg/m², 75 mg/m², 80 mg/m², 85 mg/m², 90 mg/m², 95 mg/m², 100 mg/m², 105 mg/m², 110 mg/m², 115 mg/m², 120 mg/m², 130 mg/m², 135 mg/m², 140 mg/m², 145 mg/m², 150 mg/m², 155 mg/m², 160 mg/m², 165 mg/m², 170 mg/m², 175 mg/m², 180 mg/m², 185 mg/m², 190 mg/m², 195 mg/m², or 200 mg/m².

In some aspects, the second therapeutic agents described herein may be administered at least once a day, twice a day, three times a day, four times a day, once every two days, once every three days, once a week, once every two weeks, once every three weeks, once every four weeks, once every 30 days, once every five weeks, once every six weeks, once a month, once every two months, once every three months, or once every four months in an oral, IV (intravenous) or SC (subcutaneous) dose.

The treatment methods described herein can continue for as long as the clinician overseeing the patient's care deems the treatment method to be effective. Non-limiting parameters that indicate the treatment method is effective include any one or more of the following: tumor shrinkage (in terms of weight and/or volume); a decrease in the number of individual tumor colonies; tumor elimination; and progression-free survival. Change in tumor size may be determined by any suitable method such as imaging. Various diagnostic imaging

modalities well known in the art can be employed, such as computed tomography (CT scan), dual energy CDT, positron emission tomography, ultrasound, CAT scan and MRI. In some aspects, a combination therapy of the invention is used to treat a tumor that is large enough to be found by palpation or by imaging techniques well known in the art, such as MRI, ultrasound, or CAT scan.

5 Exemplary lengths of time associated with the course of therapy include about one week; about two weeks; about three weeks; about four weeks; about five weeks; about six weeks; about seven weeks; about eight weeks; about nine weeks; about ten weeks; about eleven weeks; about twelve weeks; about thirteen weeks; about fourteen weeks; about fifteen weeks; about sixteen weeks; about seventeen weeks; about eighteen weeks; about nineteen weeks; about twenty
10 weeks; about twenty-one weeks; about twenty-two weeks; about twenty-three weeks; about twenty four weeks; about seven months; about eight months; about nine months; about ten months; about eleven months; about twelve months; about thirteen months; about fourteen months; about fifteen months; about sixteen months; about seventeen months; about eighteen months; about nineteen months; about twenty months; about twenty one months; about twenty-
15 two months; about twenty-three months; about twenty-four months; about thirty months; about three years; about four years; and about five years.

In some embodiments, Compound 2, or a pharmaceutically acceptable salt thereof, is administered for seven consecutive days, either alone as a monotherapy or in combination with one or more additional therapeutic agents (e.g., chemotherapeutic agents or biotherapeutic
20 agents).

In some embodiments, the therapeutic agents described herein can be administered for one cycle comprising seven consecutive days, fourteen consecutive days, twenty-one consecutive days, twenty-eight consecutive days, thirty-five consecutive days, forty-two consecutive days, forty-nine consecutive days, fifty-six consecutive days, sixty-three consecutive
25 days, or seventy consecutive days. In some embodiments, the administration takes place for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more cycles. In some embodiments, each new cycle begins the day after the end of the previous cycle. In other embodiments, each new cycle begins 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14
30 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 1 month, 2 months, 3 months, or 4 months after the previous cycle.

In some embodiments, each therapeutic agent can have different days in one cycle. For example, Compound 2, or a pharmaceutically acceptable salt thereof, is administered for one cycle comprising twenty-eight days, and a second therapeutic agent such as docetaxel is administered for one cycle comprising twenty-one days. In some of these embodiments, administration takes place for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21,
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22, 23, 24, 25, 26, 27, or 28 cycles. In some embodiments, administration takes place for 4 cycles, for 6 cycles, for 12 cycles, or for 24 cycles.

In some embodiments, Compound 2, or a pharmaceutically acceptable salt thereof, is administered for one cycle comprising twenty-eight consecutive days as a monotherapy. In some of these embodiments, administration takes place for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 cycles. In some embodiments, administration takes place for 4 cycles, for 6 cycles, for 12 cycles, or for 24 cycles. In some embodiments, each new cycle begins the day after the end of the previous cycle. In other embodiments, each new cycle begins 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 1 month, 2 months, 3 months, or 4 months after the previous cycle.

In certain embodiments of the invention, the patient is administered 1 mg, 2 mg, 4 mg, 6 mg, 8 mg, 12 mg, 16 mg, 32 mg, 60 mg, 120 mg or more of Compound 2 QD (once daily).

In some aspects, the first therapeutic agent (*e.g.*, Compound 2) may be administered simultaneously (*i.e.*, in the same medicament), concurrently (*i.e.*, in separate medicaments administered one right after the other in any order) or sequentially in any order with the second (or more) therapeutic agent. Sequential administration is particularly useful when the therapeutic agents are in different dosage forms (*e.g.*, Compound 2 is a tablet or capsule and the second therapeutic agent is a sterile liquid) and/or are administered on different dosing schedules. For example, Compound 2 is administered once daily and the second therapeutic agent is administered less frequently, such as once weekly, once every two weeks, or once every three weeks.

In some aspects, the second therapeutic agent in the methods described herein can be administered using the same dosage regimen (dose, frequency and duration of treatment) that is typically employed when the agent is used as monotherapy for treating the same cancer. In other aspects, the subject may receive a lower total amount of at least one of the therapeutic agents than when the agent is used as monotherapy, *e.g.*, smaller doses, less frequent doses, and/or shorter treatment duration.

In some aspects, the treatment methods and uses described herein may be used prior to or following surgery to remove a tumor and may be used prior to, during or after radiation therapy.

In some aspects, the treatment methods and uses described herein is administered to a patient who has not been previously treated with a therapeutic or chemotherapeutic agent, *i.e.*, is treatment-naïve. In other aspects, the treatment is administered to a patient who failed to achieve a sustained response after prior therapy with a therapeutic or chemotherapeutic agent, *i.e.*, is treatment-experienced.

In some aspects, the subject has received a prior therapy to treat the tumor, and the tumor is relapsed, metastatic, or refractory.

In some embodiments, the treatment is administered to a subject who failed to achieve a sustained response after prior therapy with another PRMT5 inhibitor. In some embodiments, the treatment is administered to a subject who have developed a splicing factor mutation and become resistant to a prior therapy with another PRMT5 inhibitor.

5 In some embodiments, the subject is premedicated with other therapeutic agents (e.g., corticosteroids such as dexamethasone) prior to the treatment methods and uses described herein.

Encompassed by the invention provided herein also include combination therapies that have additive potency or an additive therapeutic effect while reducing or avoiding unwanted or adverse effects. The invention also encompasses synergistic combinations where the therapeutic efficacy is greater than additive, while unwanted or adverse effects are reduced or avoided. In certain aspects, the methods and compositions provided herein permit treatment or prevention of diseases and disorders wherein treatment is improved by an enhanced anti-tumor response using lower and/or less frequent doses of at least therapeutic agent in a combination therapy to at least one of: i) reduce the incidence of unwanted or adverse effects caused by the administration of the therapeutic agents separately, while at least maintaining efficacy of treatment; ii) increase patient compliance, and iii) improve efficacy of the anti-tumor treatment.

Kits

20 The therapeutic agents of the treatment methods and uses described herein may conveniently be in the form of a kit for administration of the compositions.

In some embodiments, a kit comprises a container and a package insert. The container contains at least one dose of Compound 2, and the package insert / label comprises instructions for treating a patient for cancer and/or cancer-associated disease using Compound 2. The container may be comprised of a specific shape (e.g., vials, syringes and bottles) and/or material (e.g., plastic or glass). The kit may further comprise other materials that may be useful in administering Compound 2, such as diluents, filters, IV bags and lines, needles and syringes.

In some embodiments, a kit comprises at least a first container and a second container and a package insert. The first container contains at least one dose of Compound 2, and the second container contains at least one dose of a second therapeutic agent of the combination therapy. The package insert / label comprises instructions for treating a patient for cancer and/or cancer-associated disease using the therapeutic agents. The first and second containers may be comprised of the same or different shape (e.g., vials, syringes and bottles) and/or material (e.g., plastic or glass). The kit may further comprise other materials that may be useful in administering the therapeutic agents, such as diluents, filters, IV bags and lines, needles and syringes.

EXAMPLES

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, practice the present invention to its fullest extent. The following detailed examples describe how to prepare the various compounds and/or perform the various processes of the invention and are to be construed as merely illustrative, and not limitations of the preceding disclosure in any way whatsoever. Those skilled in the art will promptly recognize appropriate variations from the procedures both as to reactants and as to reaction conditions and techniques.

Materials and Methods

10 PRMT5:MEP50 cloning and protein production

Full length human PRMT5 was cloned into pFastBac1 (ThermoFisher), and human MEP50 (WDR77) was cloned into a modified pFastBac1 vector with an N-terminal 6xHis and TEV cleavage site (MASHHHHHHDYDGATTENLYFQGS (SEQ ID NO: 1)). Human pICIN (CLSN1A) and SMD3(SNRNPD3) were cloned into pET vectors (Novagen). The human pICIN expression vector is untagged and Smd3 expression constructs were modified with an N-terminal 6xHis and TEV cleavage site (MASHHHHHHDYDGATTENLYFQGS (SEQ ID NO: 1)). PRMT5 and MEP50 proteins were co-expressed in the Bac-to-Bac baculovirus (ThermoFisher) system using manufacturer's protocol in Sf21 cells for 72 hours in ESF-21 protein free media. Smd3 and pICLN were co-transformed into the BL21-AI strain of *E. Coli* in TB media at 37°C. Once the culture reached density, ITPG (0.4 mM) and arabinose (0.02%) was added and grown at 16°C overnight.

Cell pellets of expressed PRMT5/MEP50 complex or Smd3/pICLN complex were lysed in 50 mM Tris pH7.4, 150 mM NaCl, 10% glycerol and 0.25 mM TCEP with protease inhibitor, then stirred at 4°C for 1 hr. Lysate was centrifuged at 10,000g for 1 hour at 4°C. Supernatant was incubated with Ni-probond resin for 2 hours, resin, washed with lysis buffer containing 20 mM imidazole, and eluted with 200 mM imidazole buffer. After TEV digest overnight to remove His-tags on MEP50 or pICLN, the sample was passed through Ni-probond column to remove TEV. The flow through was concentrated and further purified on a Superdex 200 26/60 column.

Co-crystal structures

Crystallization of full-length human PRMT5/MEP50 complexed with cofactor site inhibitors was performed at 13°C by hanging-drop vapor-diffusion methods. 2.5 ul of a solution of 5:1 molar ratio of inhibitor compound to PRMT5/MEP50 complex (13 mg/mL) was mixed with 2.5 ul of reservoir solution containing 13-15% (w/v) PEG3350, 0.1M MES, pH 6.5-7.5, 0.25M NaCl, and 20% (v/v) ethylene glycol. Microseeding from initial crystals produced crystals suitable for data collection. Crystals for data collection were flash-frozen in liquid N₂ using 25% (v/v) ethylene glycol in the mother liquor as a cryoprotectant and shipped for to the Advanced Photon Source IMCA-CAT beamline 17-ID at Argonne National Labs for diffraction data collection. Diffraction data were processed with autoPROC from Global Phasing and structure solution and refinement

were done with BUSTER using the published structure of human PRMT5/MEP50 (PDB ID 4GQB) as the initial model. Model building was done with COOT (see, e.g., Emsley, P. *et al.*, Acta Crystallogr D Biol Crystallogr, 60(Pt 12 Pt 1):2126-32 (2004)).

5 PRMT5 Biochemical assay

PRMT5/MEP50 protein complex was combined with H4(1-21) peptide (SGRGKGGKGLGKGGAKRHRKV (SEQ ID NO: 2)) or SmD3 (99-119) peptide (KAAILKAQVAARGRGRGMGRG (SEQ ID NO: 3)) in PRMT5 assay buffer (50 mM Tris pH 8.5, 50 mM NaCl, 5 mM MgCl₂, 1 mM EDTA, 1 mM TCEP or DTT) and 44 µl was added to the microtiter plate containing compound. S-Adenosyl-L-methionine (SAM) was prepared by combining ³H labeled SAM with unlabeled SAM in PRMT5 assay buffer such that the total SAM concentration was 5 times the desired SAM concentration for the assay, the concentration of ³H labeled SAM was 0.5 µM. The reaction was initiated by adding 10 µl of SAM stock to the microtiter plate. The final reaction conditions were 0.75 - 3 nM PRMT5/MEP50 complex, 200 nM H4 peptide or 250 nM SmD3 peptide and various SAM concentrations typically between 1 and 10 µM. Following a 25-60 minute incubation at room temperature, the reaction was stopped with the addition of 100 µL of 20% TCA. The ³H-peptide product was captured using a 96-well filter plate (MSIPN4B, Millipore) and washed 5 times with PBS buffer. Scintillation fluid (100 µl) was added to the dried filter plate and ³H-methyl peptide product was quantified using a liquid scintillation counter.

For SAM K_m^{app} and k_{cat}^{app} determinations, enzyme activity was monitored as a function of SAM concentration and a fixed (100 nM) concentration of SmD3 (99-119) peptide substrate. Peptide K_m^{app} is estimated to be 10 nM. Enzyme concentration was 0.75 nM for WT complex, 1.6 nM for F327L complex and 1.1 nM for M420T complex. Cold SAM was mixed with ³H SAM, such that the final SAM concentration was 5x the highest SAM concentration desired for the titration, followed by 2x serial dilution in assay buffer or into assay buffer with 0.5-1 µM ³H SAM. For WT and F327L enzyme complexes, the highest SAM concentration used was 2.5 µM (2.375 µM cold SAM, 0.125 µM ³H SAM). For the M420T mutant complex, the top SAM concentration used was 20 µM (19 µM cold, 1 µM hot). Data were fit to the Michaelis-Menton equation using Prism software (GraphPad Software, Inc.) SAM specific activity (cpm/pmol of SAM) was calculated by spotting a small amount of the 5x SAM solution onto a wet filter after filtration was completed and processing alongside the reaction wells. Active enzyme concentration was estimated by active site titration using multiple tight binding inhibitors, including Compound 1 and Compound 2.

For inhibitor K_i determinations, compounds were solubilized in DMSO and serially diluted 3-fold into 100% DMSO at a concentration 50-fold greater than the desired final assay concentration. Following dilution, 1 µl was added to an empty 96-well microtiter plate prior to addition of enzyme or substrates. Compounds with IC₅₀ values below 10 nM were tested at 10 µM SAM concentration, all others were tested at a final SAM concentration of 1 µM.

K_i values were determined by fitting the data to the tight binding competitive inhibition equation using proprietary software. For EPZ015666, inhibition was measured at saturating concentrations ($33 \times K_m$) of SAM and K_i values were estimated by fitting the data to the equation for competitive inhibition with respect to the peptide substrate. The lower limit of quantitation for the assay with WT PRMT5/MEP 50 was conservatively estimated to be 5 pM based on 0.75 nM active enzyme concentration and $[S]/K_m$ for SAM = 33.

For mechanism of inhibition studies with Compound 1, K_i^{app} values were determined as multiple different SAM concentrations using the same assay as for K_i determination except that the data were fit to the Morrison equation for tight binding inhibitors without the competitive inhibition component, generating K_i^{app} (Copeland, 1996). K_i^{app} ($=IC_{50}$) was plotted as a function of $[SAM]/K_m$. For Compound 2, the K_i cannot be accurately determined, particularly at low $[SAM]/K_m$ ratios. The off-rate for the enzyme inhibitor complex is about 10 hours so equilibrium K_i^{app} values cannot be determined in a 2 hour assay. In order to investigate whether Compound 2 is competitive against SAM, an experiment was set up so that the on-rate of Compound 2 would be decreased by the presence of competing substrate. PRMT5/MEP50 (0.75 nM) was incubated with 0.3-30 μ M SAM for 15 minutes at RT. The enzyme:SAM mix was added to serially diluted Compound and the reaction was immediately initiated with SMD3 peptide (250 nM). K_i apparent values were determined by fitting the data to the Morrison equation for tight binding inhibitors. K_i apparent was plotted as a function of $[S]/K_m$ for SAM.

SPR (Surface Plasmon Resonance)

Biacore T200 instrument was desorbed and loaded with a Series S Sensor Chip SA. Biotinylated PRMT5-MEP50 complex was diluted to 50 μ g/mL with assay buffer (25 mM HEPES, 150 mM NaCl, 1 mM TCEP, 0.02 % Tween-20, 1% DMSO, pH 7.4) and injected into ligand channel at a flow rate of 5 μ L/min and a contact time of 5 min at 25 $^{\circ}$ C. Approximately 3000 RU of PRMT5-MEP50 complex was captured on the streptavidin-coated biosensor chip. The functionalized surface was then equilibrated with assay buffer for approximately 2 hours. An un-functionalized channel was used as a reference surface for binding kinetic analysis. A five-fold, 5-point serial dilution of test compounds was set-up in a deep 96-well microplate (Greiner; Cat # 780201). Binding kinetics was measured at 25 $^{\circ}$ C or 37 $^{\circ}$ C in a single-cycle kinetics format by injecting serial dilution of compounds onto reference and ligand channel at a flow rate of 100 μ L/min and association time of 200 seconds. Compound dissociation was monitored for 3000-5000 seconds. Two buffer blanks were also run in a single-cycle kinetics format before the compound run for double referencing. No additional regeneration was used. DMSO calibration curve was obtained before and after compound analysis by injecting 0-2% of DMSO in running buffer. Data analysis was performed using Biacore T200 analysis software. The double-referenced and solvent-corrected data was fit to 1:1 Langmuir model to obtain binding constant

(K_D) and binding kinetics (k_{on} and k_{off}) information. The adequateness of the fit was judged by χ^2 values (lower than 5% of the R_{max}) and the randomness of residue distribution. The raw kinetic data as well as the fit were then imported into Graphpad Prism for visualization.

5 Pharmacokinetic assay in rats

Compound 2 was administered to male Wistar-Han rats (n = 2/group) at the doses of 2 mg/kg intravenously and 10 mg/kg orally. Blood samples were collected at 0.033 (intravenous only), 0.083, 0.25, 0.5, 1, 2, 4, 7 and 24 hours postdose to quantify plasma concentrations of Compound 2 by liquid-chromatography tandem mass spectrometry. Pharmacokinetic parameters including maximal plasma concentration (C_{max}), time to C_{max} (t_{max}), area under the plasma concentration-time curves (AUC) and half-lives ($t_{1/2}$), were determined by non-compartment pharmacokinetic analysis.

Cell culture and reagents

15 A2780 cells (Sigma), NCI-H441, NCI-H1975 (ATCC CRL-5908) were maintained in RPMI1640 + 10% FBS + 1X Pen/Strep. A427 cells were maintained in EMEM + 10% FBS + 1X Pen/Strep. Additional NSCLC cell lines used in the proliferation assays were grown in manufacturer recommended growth media.

20 RBM10 stable clone generation

NCI-H1975 cells were seeded in 6-well plate at 10^6 cells/well (RPMI1640 + 10% FBS + 1% Antibiotic-Antimycotic (ThermoFisher, Cat# 5240096)) and incubated at 37°C, 5% CO₂ overnight. Prior to transfection, medium was replaced with 1.5 ml/well fresh RPMI medium without antibiotics. Transfection was performed using Lipofectamine 2000 (ThermoFisher 1901464) following manufacturer's recommended protocol with 3 ug RBM10 cDNA plasmid (Origene, Cat# RC200150, NM_005676). Transfection was stopped by feeding cells with fresh medium in the presence of 800 ug/ml G418 (ThermoFisher, Cat No 10131-027) and continued culture for 8 days in the 6-well plate before further expansion and selection. The transfected cell pool was maintained in complete RPMI1640 medium plus 800 ug/ml G418 with medium change every 4 days. For stable clone generation, cells from each transfected pool were seeded in three 150 mm dishes at 2000 cells/dish and cultured with complete RPMI1640 medium plus 800 ug/ml G418 until the appearance of colonies. Individual clones were picked up and expanded in complete RPMI1640 medium with G418. Clones which displayed RBM10 protein expression, similar to endogenous RBM10 in NSCLC cell lines were selected for further studies.

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Generation of resistant cell lines

A2780 cells were seeded at 2.5×10^5 cells in a T75 flask and treated with GI50 doses of Compound 1 (1 nM) and EPZ015666 (450 nM). Media was refreshed 2X per week and cells

counted 1X per week to monitor proliferation. Once proliferation reached a plateau, compound concentrations were increased incrementally for a total of 40 weeks. The final concentrations of each compound used at the final resistance stage was 14 nM for Compound 1 and 15 μ M for EPZ015666. At week 40, resistant cell lines were analyzed by whole exome sequencing for acquired mutations at WuXi NextCode (Supplemental data SX).

Cellular Fractionation

Cells were washed with cold PBS, resuspended in 4x volume of swelling buffer (10 mM Tris pH 8.0, 1.5 mM $MgCl_2$, 10mM KCl, protease inhibitor cocktail). Following a 15 minute incubation on ice, 1x swelling buffer + 1% Triton X-100 was added and mixed. Lysate was centrifuged at 1000g for 10 minutes at 4°C. Supernatant containing cytoplasmic fraction was put in a new tube and NaCl added to a final concentration of 200 mM. The nuclear pellet was washed 5x with swelling buffer + 0.2% Triton X-100 by vortexing and centrifuging pelleted at 1000g for 3 minutes at 4°C, then suspended in nuclear extraction buffer (10 mM Tris pH 8.0, 1.5 mM $MgCl_2$, 10 mM KCl, 400 mM NaCl, 0.4% Triton X-100, protease inhibitor cocktail) and vortexing for 30 minutes at 4°C. Nuclear lysate was centrifuged at 16,000g for 15 minutes at 4°C and supernatant removed.

Immunofluorescence

Cells were grown on chamber slides, fixed in ice-cold methanol for 10 minutes at -20°C, then washed twice with ice-cold PBS. Slides were incubated with PBS + 0.25% Triton X-100 for 10 minutes at room temperature, then washed three times in PBS. Slides were blocked using 1% BSA in PBST (PBS + 0.1% Triton) for 60 minutes at room temperature. Blocking buffer was removed, replaced with PRMT5 antibody diluted 1:100 in 1% BSA in PBST and incubated at 4°C overnight. PRMT5 antibody was removed and slides washed three times in PBS for 5 minutes each. Fluorochrome-conjugated antibody was diluted in 1% BSA in PBST and slides incubated at room temperature for 1 hour in the dark. Secondary antibody was removed and samples washed three times in PBS for 5 minutes each in the dark. Chamber was removed and slides were coverslipped with Prolong Diamond Antifade Mountant with DAPI prior to imaging.

Western blotting

Cells were lysed in RIPA buffer + protease/phosphatase inhibitor and cleared by centrifugation (14,000 rcf for 20 minutes at 4°C). Histones were purified using the Histone Purification Kit (Active Motif). Protein concentration was determined using the bicinchoninic acid assay (BCA) or Bradford method. Protein lysates were loaded at 20 μ g per lane and histones at 500 ng per lane. Proteins were separated on 4-12% bis-tris polyacrylamide gels in MOPS running buffer and transferred to nitrocellulose membranes. Membranes were blocked in Odyssey blocking buffer for one hour at room temperature, then incubated with primary antibody overnight

at 4°C. Primary antibody dilutions were as follows: anti-SDMA, H3R8me2s, H2AR3me2s, H4R3me2s, H3K27me3, PARP, cleaved PARP, p53, p21, and cleaved caspase-3 at 1:1000, anti-tubulin, total histone H3 and beta-actin (1:5000), total histone H4 (1:10,000). Membranes were washed 5X with PBS + Tween 20 (0.1%), and then incubated with secondary antibody (anti-rabbit or anti-mouse at 1:20,000) for 1 hour at room temperature. Membranes were washed 5X in PBS + Tween 20 (0.1%) and imaged using a LI-COR Odyssey CLx imager. Bands were quantified using the LI-COR Image Studio software.

Senescence assays

A549 cells were seeded in 6-well plates (10,000 cells/well). Media and compound were refreshed every 3-4 days, and cells were passaged upon reaching ~90% confluence. After 10 days of treatment, cells were fixed in 4% paraformaldehyde (w/v) for 15 minutes at room temperature and stained for SA- β -Galactosidase positivity with the Senescence β -Galactosidase Staining Kit (CST9860).

Proliferation assays

NSCLC cells were seeded in 96 well plates in recommended culture media and incubated overnight at 37°C, 5% CO₂. The following day, fresh media with compound (diluted in DMSO) was added and cells incubated at 37°C, 5% CO₂ for 7 days with media/compound refreshed at Day 3-4. On day 7, culture media was removed and cells were lysed in Cell Titer Glo reagent for 10 min and read on an Envision plate reader with luminescence filter. Alternatively, CyQuant reagent was added to the plates at Day 7, the plate incubated at 37°C for 1 hour and read on plate reader with fluorescent filter.

For lowest cytotoxic concentration determination, A427 or NCI-H441 cells were seeded at 100,000 cells per well in 1 ml in a 12-well plate and incubated overnight at 37°C, 5% CO₂. The following day, DMSO or Compound 2 was added and incubated at 37°C, 5% CO₂ for 3-4 days. At each timepoint, cells were removed with 100 μ l trypsin and neutralized by adding 1.9 ml culture media. 1 ml of the cell dilution was re-plated for continued incubation at 37°C, 5% CO₂ and the other half counted using a Vi-Cell cell counter. Cell counts were taken every 3-4 days up to 20 days to determine the lowest dose that demonstrated cytotoxicity.

Cell cycle analysis

Cells were seeded at appropriate densities to allow for growth of 80-90% confluency by Day 5. Cells were treated in dose response with Compound 2 with DMSO as a control. Nocodazole treatment was used as a control for G2/M arrest and thymidine block was used as a G1 arrest control for each cell line. Cells were treated with Compound 2 for either 4 or 5 days, stained with propidium iodide and analyzed by flow cytometry to observe changes in the phases of the cell cycle.

RNA-seq

In preparation for RNA-seq, A427, NCI-H441 and NCI-H1975 cells were seeded in triplicate at one million cells per 10 cm tissue culture dish in recommended growth media overnight at 37C, 5% CO₂. The following day, plates were treated with either 0.1% DMSO or 30 nM Compound 2 for 72 hr. Cells were preserved in RNALater reagent and sent to WuXi NextCode for RNA isolation and sequencing at 100MM read depth. Sequencing reads were mapped to hg19 genome using STAR and quantified using RSEM. The DESeq2 program was used for differential expression analysis and alternative splicing analysis was done using rMATS version 4.02 (See, Shen, S., et al., Proc Natl Acad Sci U S A, 111(51): E5593-601 (2014)). Gene pathway analysis utilized a hypergeometric test with FDR correction (See, McMillan E.A., et al., Cell, 173(4): 864-878 e29 (2018)).

Dose response curve fitting

Dose response curves were fit as previously described (see, e.g., McMillan et al, (2018), supra, PMID 29681454).

Elastic Net Analysis

The elastic net analysis was performed as previously described using cell line AUC and EC50 data as input response vectors in which to identify predictive features. Feature datasets were extracted from the CCLE (see, e.g., Ghandi, M., et al., Nature, 569(7757): 503-508 (2019), PMID 31068700) using log2 transformed TPM data from RNA sequencing and SNP copy number profiles. Non-synonymous mutation annotations (De_novo_Start_OutOfFrame, Missense_Mutation, Nonsense_Mutation, Splice_Site, Start_Codon_SNP) were extracted from maf files using the 1,346 cell lines that were profiled with whole exome sequencing. EC50 vectors were log transformed prior to input into the elastic net. Supplementary data SX provided list elastic net calculated weights and bootstrapped frequencies. No feature reached a minimum bootstrapped frequency of .5 to be considered.

Gene Set Enrichment Analysis

Cells were dichotomized into two classes (sensitive and resistant) based on EC50 values and curve shapes. Gene set enrichment analysis was performed as previously described (see, e.g., Subramanian et al, 2009 PMID 16199517).

In Vivo Human Lung Cancer Xenograft Models

A427 and NCI-H441 human lung cancer cell lines for *in vivo* experiments were purchased from ATCC and cultured in accordance with the manufacturer's guidelines. Xenograft lines were validated via STR typing to ensure cell line integrity.

All animal procedures were approved by Pfizer's Institutional Animal Care and Use Committee (IACUC) and Crown Bio's IACUC and completed in accordance with the guidelines and regulations of the IACUC, NIH, and Animal Welfare Act.

A427 xenograft experiments were performed using 6-8-week-old female NSG mice purchased from Jackson Labs (strain name NOD.Cg-Prkdc^{scid}IL2rg^{tm1Wjl}/SzJ). Prior to inoculation, cells were tested to confirm absence of mycoplasma. In brief, 5×10^6 A427 cells (0.2 mL in 50% Matrigel, Trevigen; and 50% serum-free RPMI 1640, Gibco) were inoculated subcutaneously into the right flank of each mouse. NCI-H441 xenograft experiments were conducted at Crown Bio, Taicang, using 7-8-week-old male Nu/Nu mice purchased from Charles River Laboratories (strain name Crl:NU-Foxn1nu). 5×10^6 NCI-H441 cells (0.1 mL in 50% Matrigel, 50% PBS) were injected subcutaneously into the flank of each mouse.

Once tumors were palpable, tumor length and width were measured by calipers 2-3 times weekly. Animals were randomly assigned to experimental groups and treatment was initiated at day 0, when tumor volume reached 150 mm³ on average. Ten animals were enrolled in each treatment arm. Tumor volume was calculated by the standard formula $L \times W^2 \times 0.5$.

For all lung xenograft experiments, Compound 2 was administered daily by oral gavage at doses of 3, 10, and 30 mg/kg (vehicle: 0.5% methylcellulose (w/v) solution with 0.1% polysorbate 80 (w/v) in water). Additionally, the NCI-H441 *in vivo* experiment included an additional treatment arm of Compound 2 dosed orally at 5 mg/kg twice daily.

Effects of drug treatment on tumor volume were analyzed by two-way ANOVA, with multiple comparisons using Tukey's post-hoc test. Data were graphed and analyzed using GraphPad Prism, version 8.1.0.

The human patient-derived xenograft (PDX) models were established from cryopreserved human NSCLC tumors obtained in accordance with appropriate consent procedures. The NSCLC PDX xenografts were subcutaneously passaged *in vivo* as fragments from animal to animal in NOD scid gamma mice. Female NOD scid gamma mice 7-10 weeks of age (NSG, Stock No: 005557), were obtained from The Jackson Laboratory (Farmington, CT). Mice were housed under specific pathogen-free conditions in Tecniplast IVC Green Line IVC cages in the vivarium at Pfizer, Pearl River, NY. Mice were housed on a 12:12 light:dark cycle, with ad libitum UV-sterilized water and irradiated Purina Chow (Purina). Animals were monitored twice daily for health status. At the start of the experiments, mice weighed 18–25 grams. Compound 2 (formulated in 0.5% methylcellulose (w/v) solution with 0.1% polysorbate 80 (w/v) in water) was dosed daily via oral gavage at either 10 or 30 mg/kg.

Pharmacokinetic Analysis

Blood samples were collected at 0.5, 1, 2, 4, 7 and 24 hours post-dose to quantify plasma concentrations of Compound 2 by liquid-chromatography tandem mass spectrometry. Pharmacokinetic parameters of Compound 2 including maximal plasma concentration (C_{max}),

time to $C_{\max}$ ($t_{\max}$), area under the plasma concentration-time curves (AUC) and half-lives ($t_{1/2}$), were determined by non-compartment pharmacokinetic analysis (Watson LIMS, Thermo Electron, Philadelphia, PA).

5 *Symmetric dimethyl arginine ELISA protocol*

Flash frozen tumors were lysed in RIPA buffer with protease/phosphatase inhibitors in Lysing Matrix A tubes (MP Bio) by homogenizing using a FastPrep-24 instrument and incubating on ice for 30 minutes. Samples were diluted with lysis buffer to a final volume of 450 μ l, transferred to 1.5 ml microcentrifuge tube and sonicated in a cold water bath sonicator. Lysates were cleared
10 by centrifugation at 4° C for 20 minutes at maximum speed and the supernatant was transferred to a new 1.5ml microcentrifuge tube. Protein concentration was determined using the Pierce™ BCA Protein Assay Kit according to manufacturer guidelines. 150 ng of lysate was added to each well of a 96 well plate in a volume of 50ul in quadruplicate and plates were incubated at 4° overnight. After washing with PBS–Tween 20 (0.1%) (Biotek plate washer), wells were blocked
15 with 100 ul/well of 5% BSA in PBS at room temperature for 2 hr. A second round of washes with PBST was followed by incubation with primary antibody 50ul/well (anti-SDMA at 1:250, or SmD3 at 1:500 in 1× PBS) at 4 °C overnight. Plates were washed with PBST and 50 ul/well of secondary antibody (1:10,000) was added (anti-rabbit IgG peroxidase conjugate, CST, 7074S, diluted in 1× PBS) and incubated in the dark for 60 min at room temperature. Plates were washed with PBST
20 and 100ul chemiluminescent substrate was added to each well and incubated at room temperature shaking (500rpm) for 1 minute. Total luminescence was measured using an EnVision reader (PerkinElmer). SDMA/SMD3 ratios were calculated for each sample and then normalized to the average of the vehicle controls. These values were plotted in GraphPad Prism
7.

25

EXAMPLE 1

Identification and characterization of SAM competitive PRMT5 inhibitors

The discovery effort began with the published crystal structure of PRMT5:MEP50 complexed with the nucleoside A9145C (Antonysamy S., *et al.*, Proc Natl Acad Sci U S A, 30 109(44): 17960-52012). To minimize the synthetic complexity associated with A9145C and AdoMet analogs, a ligand truncation strategy was employed to define a minimum pharmacophore. This effort led to a key finding of adenosine (**FIG. 1**) as an efficient inhibitor of PRMT5 (K_i = 100 nM CI =70-140 nM). Determination of the PRMT5:MEP50:adenosine co-crystal structure revealed adenosine bound in the cofactor pocket, with the purine and the sugar binding
35 similar to what is observed in the A9145C co-crystal structure (**FIG. 1**). Specifically, the purine donating an H-bond to Asp419 and accepting from Met420 was observed. Additionally, both ribose hydroxyls were observed interacting with Glu392 and the 5'-hydroxyl appeared to interact with a network of crystallographic water molecules. Finally, a deep binding pocket was observed

adjacent to the 5'-carbon, providing a convenient vector for establishing additional protein/ligand interactions. An iterative protein structure and property-based design effort eventually led to incorporation of a 3-fluoro-4-chlorobenzene at the 5' position. Co-crystal structure determination showed that the dihalogenated phenyl ring efficiently filled space in the deep pocket and established an edge to face interaction with Tyr324 (**FIG. 1**). The careful consideration around protein pocket complementarity and small molecule physical properties led to Compound 1 (**FIG. 1**), a high quality in vitro and in vivo tool compound.

Compound 1 was utilized to design a structurally orthogonal, co-factor competitive series of inhibitors with diverse physicochemical properties. Further examination of the deep binding pocket associated with the Compound 1 co-crystal structure revealed opportunities to interact with Glu444. Glu444 is a catalytically important residue responsible for binding the substrate Arg guanidine terminus during the methyltransferase reaction. In order to achieve this interaction, a complete redesign of the Compound 1 deep pocket binding region was required. Two significant changes were made to the Compound 1 structure. First was the linkage between the ribose sugar and the dihalogenated phenyl ring. Guided by the Compound 1 co-crystal structure, the inventors removed the 5' carbon and attached hydroxyl, and moved the ribose oxygen from within the sugar ring to the 5' position. This modification created a phenyl ether and positioned the phenyl ring in the deep pocket such that appropriate vectors were made available to target Glu444 with basic amines. Second was the overall substitution pattern around the dihalogenated phenyl ring. With a slightly different pose of the phenyl ring buried in the deep pocket, a redesign of phenyl substituents was required to preserve efficient pocket complementarity.

Targeting Glu444 from the phenyl ring led to design of a nitrogen-containing 6-membered ring, ultimately yielding a tetrahydroisoquinoline (THIQ) system. Modeling the THIQ suggested the basic nitrogen could potentially form a charge-charge interaction with Glu444 residue. Moreover, modeling predicted the THIQ nitrogen could establish H-bonds directly with Leu437 and Glu435 backbone carbonyl oxygens. Structure-based iterative design into the deepest region of the co-factor site binding pocket led to optimal THIQ substitution. A fluorine atom para to the ether link along with an adjacent difluoromethyl group ultimately gave Compound 2 (**FIG. 1**). A co-crystal structure was solved with Compound 2 bound in the co-factor site with extension into the edge of the Arg substrate binding site (**FIG. 1**). As modeling suggested, the co-crystal structure confirmed interaction with Glu444, Leu437 and Glu435. Moreover, the deep pocket was efficiently complemented by THIQ halogen substitution. Compound 2 is a small molecule possessing a desirable balance between tight enzyme binding and drug-like physicochemical properties.

The inventors have discovered that both Compound 1 and Compound 2 are tight binding reversible inhibitors with demonstrated biochemical and cellular activity against PRMT5. Enzyme assays using full length human PRMT5/MEP50 protein complex were used to estimate inhibition constants (K_i) and to evaluate inhibitor mechanism of action. A K_i value of 11 pM (95% CI=7-17

pM) was determined for Compound 1, however, an accurate K_i for Compound 2 was unable to be determined because the potency was below the lower limit of quantitation for the assay (<5 pM, see methods). The mechanism of inhibition for Compound 1 was investigated by determining the IC_{50} at multiple concentrations of SAM substrate. A linear increase in IC_{50} was observed upon increasing SAM concentration, consistent with a competitive inhibition model. Compound 1 appears non-competitive with respect to SmD3 peptide substrate.

Due to the limitations in determining binding affinity for Compound 1 enzymatically, direct binding studies were performed to quantify binding affinity and inhibitor off-rate for Compound 2. Single cycle kinetic analysis was performed by surface plasmon resonance (SPR) (FIG. 2) yielding a K_D of 5.8 pM and an off-rate of $1.87 \times 10^{-5} \text{ s}^{-1}$. This off-rate corresponds to a half-life for the enzyme-inhibitor complex of over 600 minutes. This half-life is very long relative to the time course of a typical enzymatic assay, making determination of inhibitor mechanism of action impractical using classical methods. The SAM competition was demonstrated by pre-forming the enzyme-SAM complex at various concentrations of SAM and then initiating the reaction in the presence of various concentrations of inhibitor. The resultant IC_{50} values were plotted as a function of SAM concentration (FIG. 3), yielding a straight line with a positive slope suggestive of competitive inhibition. Given the structural similarity of Compound 2 to Compound 1 and adenosine, Compound 2 is characterized as a SAM competitive inhibitor. Superposition of Compound 2 bound to PRMT5/MEP50 with the PRMT5/MEP50 SAH structure (FIG. 4) shows Compound 2 occupying a large portion of the SAH binding pocket, further supporting a SAM competitive mechanism for Compound 2.

Selectivity studies across a panel of protein methyltransferases indicate that Compound 1 is at least one million-fold selective for PRMT5 over the other enzymes in the panel. Only DOT1L showed any activity above 20% inhibition at a dose of 10 μM . Inhibition across a panel of 40 diverse protein kinases was also tested. Six kinases showed inhibition of greater than 20% at 10 μM Compound 1 with MAP4K4 being the most inhibited at 76%. Follow-up IC_{50} values were obtained for MAP4K4, PRKACA and ROCK1. These data show that Compound 1 is at least 250,000-fold selective over these protein kinases. The high selectivity of Compound 1 for PRMT5 over other protein methyltransferases is similar to the selectivity profile observed for adenosine. Selectivity of Compound 2 at 10 μM was assessed in both protein methyltransferase and kinase selectivity panels and showed no activity above 20% inhibition.

In vivo, Compound 1 showed moderate plasma clearance and steady-state volume of distribution (~40 mL/min/kg and 3.8 L/kg, respectively) in male Wistar-Han rats following a single intravenous administration at the dose of 2 mg/kg. Oral bioavailability was moderate (~40%) in rats following a single oral administration at the dose of 10 mg/kg. Elimination half-lives for the intravenous and oral administration were 1.5 and 3.2 h, respectively, suggesting flip-flop kinetics although it might be due to the limited time points after 7 h post-dose. These results demonstrate that Compound 2 is orally available in animals.

EXAMPLE 2**Development and characterization of PRMT5 inhibitor resistant cells**

The emergence of drug resistance has been a common observation to many targeted and chemotherapies utilized in oncology. Given the novel cellular mechanisms impacted by PRMT5 inhibition, the mechanisms capable of leading to PRMT5 inhibitor acquired resistance were studied. Compound 1 demonstrates dose dependent reduction of symmetric di-methyl arginine (SDMA), and an anti-proliferative response in cancer cell line A2780. Drug resistant cells were generated by treating A2780 cells with proliferation IC₅₀ doses of the SAM competitive inhibitor Compound 1 or peptide substrate inhibitor EPZ015666 (as disclosed in Chan-Penebre, E., et al., Nat Chem Biol, 11(6): p. 432-7 (2015)), and increasing the dose as cells became resistant for a time period of 38 weeks. After 13 weeks, cells treated with EPZ015666 developed complete resistance and were insensitive to compound doses as high as 15 μ M. Interestingly, Compound 1 treated cells only developed partial resistance, evidenced by a 5-fold shift in IC₅₀ even after 38 weeks of drug treatment. Both PRMT5 inhibitor resistant A2780 cell lines maintained drug resistance following extended culture in drug free media, suggesting a stable mechanism of drug resistance had emerged.

A2780 cells resistant to Compound 1 (A2780-5800R) or EPZ015666 (A2780-5666R) were further tested for sensitivity to both compounds. As expected, A2780-5666R cells were insensitive to EPZ015666 (IC₅₀ > 10 μ M), but showed similar sensitivity as parental cells in response to Compound 1 (IC₅₀ = 2.3 nM and 3.3 nM respectively). A2780-5800R cells retained sensitivity to Compound 1 (IC₅₀ = 15.1 nM), but the IC₅₀ was shifted 5-fold compared to parental A2780 (IC₅₀ = 3.3 nM). A2780-5800R cells are as sensitive to inhibition by EPZ015666 as the parental line (IC₅₀ = 319 nM and 233 nM respectively). Overall, these data suggest the emergence of compound-selective drug resistance in cells that track with residual PRMT5 enzyme function in the presence of drug.

To identify potential genetic mechanisms of drug resistance, whole exome sequencing was performed on A2780-5800R and A2780-5666R cells. In both cell models, unique missense mutations were identified in the predicted PRMT5 coding region. A2780-5666R cells acquired a PRMT5 missense mutation, 979T>C, converting phenylalanine 327 to leucine. This mutation occurs in the binding site for EPZ015666 where modeling predicted the mutation to negatively impact compound binding (**FIG. 7**). Attempts to generate co-crystal structures of EPZ015666 bound to Phe327Leu mutated PRMT5:MEP50 were unsuccessful. A2780-5800R cells acquired a missense mutation, 1259T>C, converting methionine 420 to threonine. Co-crystal structures obtained for the Met420Thr mutated PRMT5:MEP50 bound to Compound 1 or Compound 2, highlight the interaction of the compounds with the threonine residue which is weaker than with methionine in wild type PRMT5:MEP50. Given the different binding modes (EPZ015666 binds in the peptide substrate pocket, and Compound 1 binds in the SAM site), it was hypothesized that mutations in PRMT5 may have been acquired under pressure of compound treatment.

To further characterize the impact of identified PRMT5 mutations on inhibitor sensitivity, biochemical K_i values were generated using recombinant mutated protein or wild-type PRMT5 in complex with MEP50. PRMT5 Phe327Leu enzyme demonstrated similar inhibition to Compound 1 as wild-type PRMT5, though cells are resistant to EPZ015666 treatment. In contrast, the PRMT5 Met420Thr enzyme showed similar inhibition to EPZ015666 as wild-type PRMT5, and demonstrated partial resistance to Compound 1 with a 22- fold potency shift in K_i from 11 pM to 220 pM. Taken together, these data suggest SAM-competitive PRMT5 inhibitors are less susceptible to the development of mutations that confer complete drug resistance since only conservative amino acid changes may be tolerated in the co-factor binding pocket.

EXAMPLE 3

PRMT5 inhibitor Compound 2 reduces symmetric di-methyl arginine biomarkers and demonstrates anti-proliferative activity in NSCLC cell lines

The objective of this example was to understand the cellular response on relevant PRMT5 substrates. A427 cells were treated with Compound 2 for 72 hours, and western blots on whole cell lysates were analyzed using a symmetrical di-methyl arginine (SDMA) specific antibody. Proteins detected by the anti-SDMA antibody were extracted from a 4-12% bis-tris polyacrylamide gel for identification by tryptic digest and mass spectrometry. Cell treatment with Compound 2 shows dose dependent loss of SDMA on several proteins, such as RNA splicing proteins SmB, D1 and D3, (**FIG. 5**) with a cellular IC_{50} for SDMA in A427 of 1.1nM. Due to the abundance of literature on histone arginine methylation by PRMT5, purified histones were analyzed for loss of H3R8me2s, H4R3me2s and H2AR3me2s upon treatment of cells with Compound 2 or Compound 1. In concordance with results reported with other PRMT5 inhibitors (see, e.g., Chan-Penebre 2015, *supra*), neither Compound 1 nor Compound 2 decreased methylation of arginine residues on histone proteins after 72-hour treatment. Moreover, cellular fractionation and western blot, as well as immunofluorescence, for PRMT5 protein shows strong expression of PRMT5 in the cytoplasm with little to no expression in the nucleus of several NSCLC cancer cell lines. Overall, these data suggest the primary mechanism of action of PRMT5 inhibitors is independent of direct chromatin regulation.

PRMT5 overexpression has been reported as a driver of tumor cell growth and survival in NSCLC. Consistent with genetic knockdown, Compound 2 treatment led to dose-dependent anti-proliferation effects in NSCLC cell lines at 7 days of treatment (**FIG. 6**). To better understand the mechanisms driving growth arrest, NSCLC cells were analyzed for phenotypic responses to treatment with Compound 2. Cell cycle analysis conducted at days 4 and 5 of Compound 2 treatment demonstrated mixed cell cycle effects; that A427 arrested in G1, NCI-H1975 cells arrested in G2/M, and while NCI-H441 showed asynchronous cell cycle arrest (**FIG. 7**). A427 cells also had a modest induction of apoptosis, as measured by increased cleaved PARP and cleaved caspase-3 protein expression (**FIG. 8**), while NCI-H1975 and NCI-H441 cells did not

increase markers of cell death (data not shown) in response to Compound 2 treatment. A549 cells responded to Compound 2 treatment by inducing senescence, evidenced by the cell morphology changes and β -galactosidase staining (**FIG. 9**). Interestingly, A549 and A427 are wild type for p53, but do not respond with a strong apoptotic response as has been observed in
5 p53 wild type lymphoma cells (Gerhart-GSK). These results highlight the complexity of phenotypic effects of Compound 2 treatment and demonstrate both cytotoxic and cytostatic cellular responses in NSCLC cells.

EXAMPLE 4

10 **Compound 2 inhibition in NSCLC impacts alternative splicing pathways**

The objective of this example was to understand the molecular mechanisms of Compound 2 in NSCLC. Three cell lines were treated with drug and analyzed by RNA-seq. Differential gene expression was analyzed in A427, NCI-H441, and NCI-H1975 cells after treatment with Compound 2. Overall, 277 genes were differentially regulated ($FC > 1.5$; $FDR < 5\%$) by
15 Compound 2 treatment in all three cell lines. For the common upregulated genes, the top pathways showed enrichment in several pathways involved in RNA splicing, as well as cell migration. The genes commonly downregulated after Compound 2 treatment enriched in metabolic pathways. Additional gene sets upregulated (metabolism pathways, growth factor signaling, cellular senescence and inflammatory response) and downregulated (cell cycle, E2F
20 transcription and Type I interferon pathways) in individual cell lines, suggest certain mechanisms influenced by Compound 2 treatment may depend on cellular context.

PRMT5 is an important regulator of pre-mRNA splicing and genetic or pharmacological inhibition has previously been shown to lead to changes in alternative splicing of specific genes. To understand the effects of Compound 2 treatment on cellular splicing, the RNA-seq data was
25 analyzed for alternative splicing changes using the rMATS algorithm (see, e.g., Wang, Y., *et al.*, EMBO Mol Med, 5(9): p. 1431-42 (2013)). Compound 2 treatment increased exon skipping and intron retention with minimal impact on other types of alternative splicing events. Overlap of the skipped exons across the three cell lines suggests a set of pre-mRNA splicing events consistently dependent on PRMT5 function in NSCLC. A conserved set of genes with skipped exons was
30 observed upon PRMT5 inhibitor treatment with 577 exon skipping events (differential percent spliced-in (ΔPSI) > 0.1 ; $FDR < 1.0\%$) shared by all three cell lines, and 1671 shared by at least two cell lines. Gene ontology analysis of the genes with skipped exons shows enrichment for microtubule organization, cell cycle and DNA repair pathway. The number of cassette exons that were increased upon Compound 2 treatment was lower compared to skipped exons, with 130
35 events in common across the three cell lines. Pathway analysis for higher cassette exon inclusion identified genes involved in cell cycle and RNA splicing pathways. These results highlight the important function of PRMT5 in the regulation of pre-mRNA splicing and suggest a unique set of genes highly dependent on PRMT5 function for post-transcriptional regulation.

EXAMPLE 5**Compound 2 demonstrates tumor growth inhibition in splicing mutant NSCLC**

The objective of this example was to further validate the anti-tumor effects of the Compound 2 treatment. Tumor growth inhibition studies were conducted in two mouse xenograft models of NSCLC. Compound 2 showed significant tumor suppression as an orally administered single agent in 2 splicing factor mutant NSCLC (A427 and NCI-H441) xenograft models. Compound 2 demonstrated dose dependent tumor growth inhibition (TGI) in the A427 (RBM10^{I348N}) model at Day 44, of 52.1% at 3 mg/kg once daily, 74.9% at 10 mg/kg once daily and 100.6% at 30 mg/kg once daily doses (**FIG. 10**). Modulation of SDMA in the A427 tumors was evaluated via ELISA at end of study with reductions of 66.4% to 79.8% compared to vehicle controls (**FIG. 11**). In the NCI-H441 (U2AF1^{S34F}) model, Compound 2 demonstrated dose dependent tumor growth inhibition (TGI) at Day 36 of 55.5% at 5 mg/kg BID, 14.9% at 3 mg/kg once daily, 46.6% at 10 mg/kg once daily and 87.2% at 30 mg/kg once daily doses (**FIG. 12**). SDMA levels were assessed in the NCI-H441 tumors was evaluated via ELISA at end of study with reductions compared to control from 70.6% to 85.9% for the once daily doses (**FIG. 13**). Compound 2 was well tolerated in both TGI studies with minimal body weight loss (**FIG. 12** and **FIG. 15**).

Following repeated oral administration of Compound 2 to mice bearing xenograft tumors with NCI-H441 at the doses of 3, 10 and 30 mg/kg, Compound 2 was rapidly absorbed with t_{max} of 1 to 2 hours. Mean unbound C_{max} ranged from 28 to 333 nM at the doses of 3 to 30 mg/kg whereas mean unbound AUC were 165 to 1266 nM·h/mL. Increases in oral exposures were dose-proportional at the doses tested. Unbound C_{ave} (AUC divided by a dosing interval of 24 hours) was 2.3 to 17.7 fold higher than the lowest cytotoxic concentration of 3 nM in NCI-H441. Plasma concentrations of Compound 2 in mice bearing xenograft tumors with A427 at the doses of 3, 10 and 30 mg/kg were comparable to those in xenograft models with NCI-H441. Unbound C_{ave} was 0.51 to 4.3 fold higher than the lowest cytotoxic concentration of 10 nM in A427.

To further validate the anti-tumor effects of the Compound 2 treatment in patient derived xenograft (PDX) models with copy number loss of the RBM10 gene, tumor growth inhibition studies were conducted in three mouse models of NSCLC with loss of RBM10. Compound 2 showed significant tumor suppression as an orally administered single agent in 3 NSCLC RBM10 loss PDX models (NSX-26183, NSX-26130 and NSX-26109) xenograft models with dose dependent tumor growth inhibition (**FIGS. 16-18**).

EXAMPLE 6**Splicing dysregulation is associated with NSCLC sensitivity to Compound 2 inhibition**

The objective of this example is to identify molecular features influencing response to Compound 2 in NSCLC. A 7 day proliferation screen across a panel of NSCLC cell lines was

conducted. Cell lines exhibited a range of sensitivities to Compound 2 with 54% of the cell lines demonstrating GI50 at ≤ 50 nM (**FIG. 19**), a dose representing IC90 of SDMA biomarker modulation. To facilitate analysis of cellular biomarkers predictive of response to Compound 2 in lung cancer in an unbiased manner, NSCLC cell proliferation screening data using multivariate elastic net analysis was analyzed. Molecular association analysis of genetic features, including mutations and copy number amplification and deletion, did not identify any statistically significant combination of features associated with inhibitor response in NSCLC. Previous studies have identified several genetic factors influencing cellular response to PRMT5 genetic loss or inhibitors, including MTAP deletion, MYC expression, p53 status, and CLSN1A to R1OK1 expression ratio. Although shRNA knockdown of PRMT5 has been shown to be synthetic lethal with MTAP deletion, only one cell line in the panel is reported to have the genetic loss, so a relationship between MTAP and Compound 2 sensitivity could not be determined. Additionally, no significant association between p53 status and sensitivity to PRMT5 inhibitors in NSCLC ($p=0.26$) was observed.

Cell line gene expression was analyzed for pathways associated with sensitivity and resistance to PRMT5 inhibitor treatment in NSCLC. Gene Set Enrichment Analysis (GSEA) (see, e.g., Subramanian, A., *et al.*, Proc Natl Acad Sci U S A, 102(43): 15545-50 (2005)) identified MYC, cell cycle, and DNA repair pathways positively associated with sensitivity to PRMT5 inhibition at FDR < 2% (**FIG. 20**). Negatively associated pathways were much less statistically significant at FDR > 25%. Target genes of MYC include many RNA processing proteins, and it has been hypothesized that MYC overexpression leads to increased dependency on core splicing machinery. Since proteins important in splicing are known substrates of PRMT5, and mutations in many splicing regulators are reported to influence tumorigenesis, global alternative splicing patterns associated with sensitivity to Compound 2 in NSCLC was analyzed. Lower total cassette exons, decreases in 5' and 3' splice site events, and increases in retained introns were observed in sensitive cell lines compared to more resistant cells ($FDR < 0.05$; $\Delta PSI > 0.1$), suggesting that basal cell line-specific alternative splicing patterns may be predictive of response (**FIG. 21**). Several genes associated with RNA processing have been identified with potential splicing factor driver mutations in tumors, but individual gene mutations are found in relatively low frequency and are often lineage specific. In NSCLC, the most common splicing factor mutations are RBM10 and U2AF1, which are represented at approximately 11% and 6% respectively, in tumors (TCGA, Seiler, M., *et al.*, Cell Rep, 23(1): p. 282-296 e4 (2018)). Using the CCLE mutation annotation for RBM10 and U2AF1 in cancer cell lines, a t-test showed significance of RBM10 mutations in the NSCLC cell lines most sensitive to the Compound 2 inhibition (**FIG. 22**). U2AF1 mutations did not show a statistically significant association with sensitivity to Compound 2 ($p = 0.46$).

To further investigate the association of RBM10 mutations with Compound 2 response in NSCLC cell lines, western blots for RBM10 was performed. The data showed that cell lines with reported frameshift mutations (NCI-H1975, NCI-2291 and NCI-H1944) have undetectable

amounts of RBM10 protein compared to RBM10 wild type cells, while cell lines such as A427 and NCI-H2286 which contain RBM10 missense mutations (I348N and A552V) have lower protein expression compared to wild type cells (**FIG. 22**).

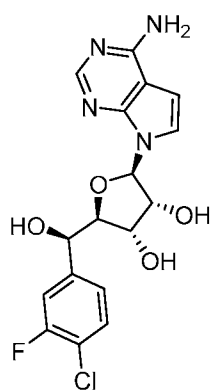
Since the frameshift mutation in NCI-H1975 causes a loss of RBM10 protein, these cells
5 were utilized to evaluate the impact of RBM10 mutants on the Compound 2 sensitivity. NCI-H1975 cells were transfected with full length RBM10 cDNA, and three clones were identified that expressed RBM10 protein at levels similar to a wild type (NCI-H460) cell line (**FIG. 23**). Analysis of alternative splicing comparing RBM10 cDNA rescued NCI-H1975 cells to parental cells shows significant increases in cassette exon inclusion with minimal impact to other types of alternative
10 splicing as is predicted for the role of RBM10 in this specific splicing regulation. In a 7 day proliferation assay with Compound 2 treatment re-expression of RBM10 decreased the potency of Compound 2 and increased the maximum total response in NCI-H1975 cells compared to the RBM10 mutant parental cells, suggesting RBM10 loss of function impacts response to Compound 2 in NSCLC (**FIG. 21**).

15 * * *

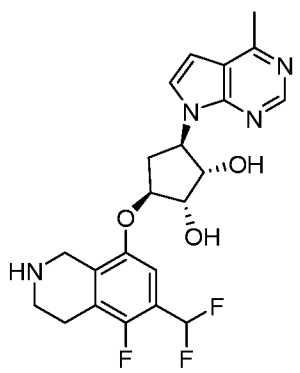
Modifications may be made to the foregoing without departing from the basic aspects of the invention. Although the invention has been described in substantial detail with reference to one or more specific embodiments, those of ordinary skill in the art will recognize that changes may be made to the embodiments specifically disclosed in this application, and yet these
20 modifications and improvements are within the scope and spirit of the invention.

We claim:

1. A method of treating cancer in a subject comprising administering to the subject in need thereof a therapeutically effective amount of a S-adenosylmethionine(SAM)-competitive Protein Arginine Methyltransferase 5 (PRMT5) inhibitor, wherein the subject has a splicing factor mutation.
2. The method of claim 1, wherein the SAM-competitive PRMT5 inhibitor is (2S,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((R)-(4-chloro-3-fluorophenyl)(hydroxy)methyl)tetrahydrofuran-3,4-diol (Compound 1) having the structure:



or a pharmaceutically acceptable salt thereof, or (1S,2S,3S,5R)-3-((6-(difluoromethyl)-5-fluoro-1,2,3,4-tetrahydroisoquinolin-8-yl)oxy)-5-(4-methyl-7H-pyrrolo[2,3-d]pyrimidin-7-yl)cyclopentane-1,2-diol (Compound 2) having the structure:

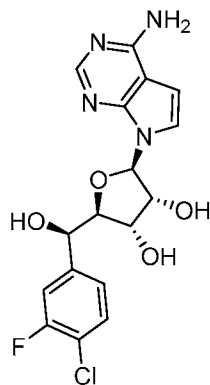


or a pharmaceutically acceptable salt thereof.

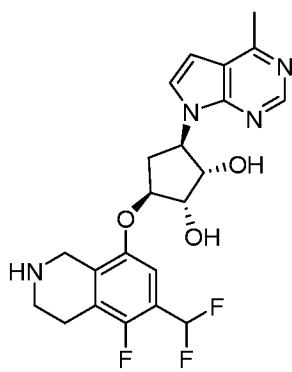
3. The method of claim 2, wherein the SAM-competitive PRMT5 inhibitor is Compound 2 or a pharmaceutically acceptable salt thereof.

4. The method of any one of claims 1-3, wherein the subject has an existing splicing factor mutation or has developed a splicing factor mutation during the course of a prior cancer treatment.
5. The method of any one of claims 1-4, wherein the splicing factor mutation is in a gene selected from the group consisting of RBM10, U2AF1, SF3B1, SRSF2, SF3A1, U2AF2, ELAVL2, FUBP1, and AQR.
6. The method of any one of claims 1-5, wherein the splicing factor mutation is a loss of function mutation.
7. The method of claim 5 or 6, wherein the splicing factor mutation in RBM10 is selected from the group consisting of G840fs, I348N, E705K, A695fs, P45L, I696fs, A552V, R171Q, G218C, Q481L, T694I, W723C, S846L, G935C, G961V, and H609fs.
8. The method of any one of claims 1-7, wherein the method further comprises administering a therapeutically effective amount of a second therapeutic agent.
9. The method of any one of claims 1-8, wherein the cancer is selected from the group consisting of lung cancer, bone cancer, pancreatic cancer, skin cancer, head or neck cancer, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, anal cancer, stomach cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, esophagus cancer, small intestine cancer, cancer of the endocrine system, thyroid gland cancer, parathyroid gland cancer, adrenal gland cancer, sarcoma of soft tissue, urethra cancer, penis cancer, prostate cancer, chronic or acute leukemia, lymphocytic lymphomas, bladder cancer, kidney or ureter cancer, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain stem glioma, and pituitary adenoma.
10. The method of claim 9, wherein the cancer is non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), esophageal cancer, endometrial cancer, cervical cancer, bladder cancer, urothelial carcinoma, or pancreatic cancer.
11. The method of any one of claims 1-10, wherein the cancer is relapsed, refractory, or metastatic.
12. Use of a compound in the manufacture of a medicament for the treatment of a subject having a cancer, wherein the subject has a splicing factor mutation, and wherein the compound is a SAM-competitive PRMT5 inhibitor.

13. The use of claim 12, wherein the SAM-competitive PRMT5 inhibitor is (2S,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((R)-(4-chloro-3-fluorophenyl)(hydroxy)methyl)tetrahydrofuran-3,4-diol (Compound 1) having the structure:

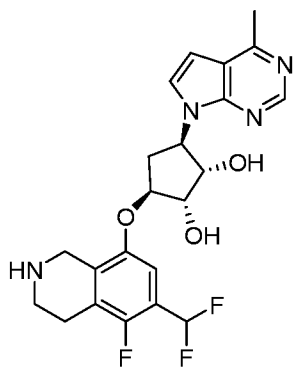


or a pharmaceutically acceptable salt thereof, or (1S,2S,3S,5R)-3-((6-(difluoromethyl)-5-fluoro-1,2,3,4-tetrahydroisoquinolin-8-yl)oxy)-5-(4-methyl-7H-pyrrolo[2,3-d]pyrimidin-7-yl)cyclopentane-1,2-diol (Compound 2) having the structure:



or a pharmaceutically acceptable salt thereof.

14. Use of a compound in the manufacture of a medicament for the treatment of a subject having a cancer, wherein the subject has a splicing factor mutation, and wherein the compound is (1S,2S,3S,5R)-3-((6-(difluoromethyl)-5-fluoro-1,2,3,4-tetrahydroisoquinolin-8-yl)oxy)-5-(4-methyl-7H-pyrrolo[2,3-d]pyrimidin-7-yl)cyclopentane-1,2-diol (Compound 2) having the structure:



or a pharmaceutically acceptable salt thereof.

15. The use of any one of claims 12-14, wherein the splicing factor mutation is in a gene selected from the group consisting of RBM10, U2AF1, SF3B1, SRSF2, SF3A1, U2AF2, ELAVL2, FUBP1, and AQR.

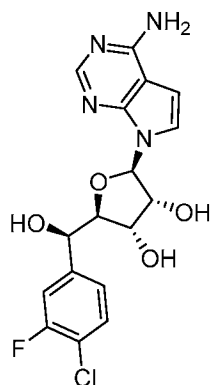
16. The use of claim 15, wherein the splicing factor mutation is a loss of function mutation.

17. The use of claim 15 or 16, wherein the splicing factor mutation in RBM10 is selected from the group consisting of G840fs, I348N, E705K, A695fs, P45L, I696fs, A552V, R171Q, G218C, Q481L, T694I, W723C, S846L, G935C, G961V, and H609fs.

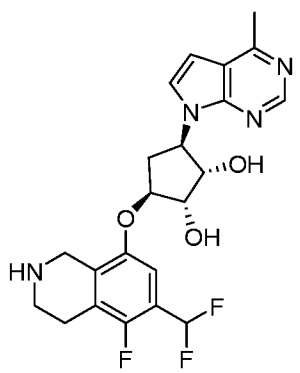
18. The use of any one of claims 12-17, wherein the cancer is non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), esophageal cancer, endometrial cancer, cervical cancer, bladder cancer, urothelial carcinoma, or pancreatic cancer.

19. A medicament comprising a therapeutically effective amount of a SAM (S-adenosylmethionine)-competitive PRMT5 (Protein Arginine Methyltransferase 5) inhibitor for use in treating cancer in a subject having a splicing factor mutation.

20. A medicament comprising a therapeutically effective amount of (2S,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((R)-(4-chloro-3-fluorophenyl)(hydroxy)methyl)tetrahydrofuran-3,4-diol (Compound 1) having the structure:



or a pharmaceutically acceptable salt thereof, or (1S,2S,3S,5R)-3-((6-(difluoromethyl)-5-fluoro-1,2,3,4-tetrahydroisoquinolin-8-yl)oxy)-5-(4-methyl-7H-pyrrolo[2,3-d]pyrimidin-7-yl)cyclopentane-1,2-diol (Compound 2) having the structure:



or a pharmaceutically acceptable salt thereof, for use in treating cancer in a subject having a splicing factor mutation.

1/23

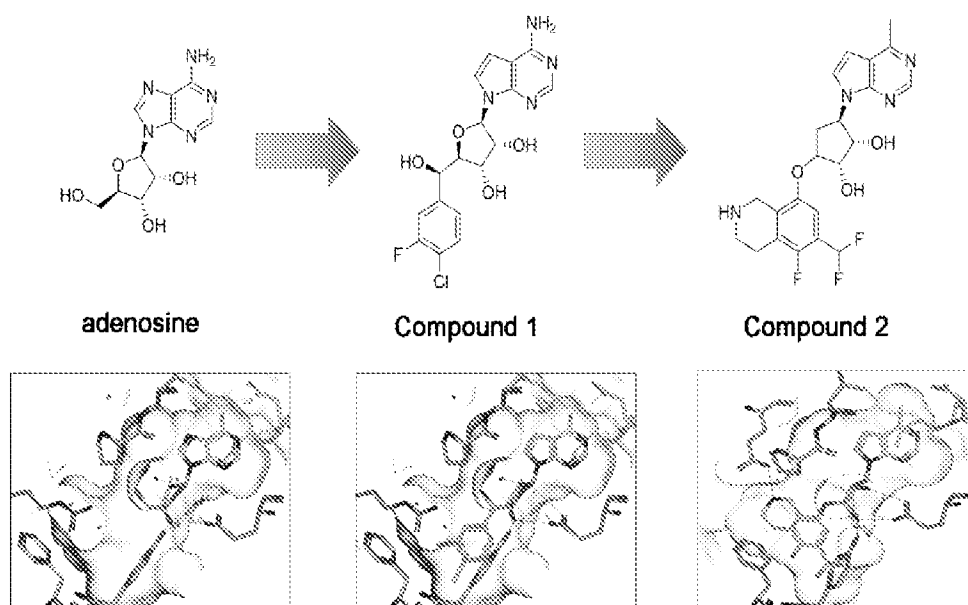
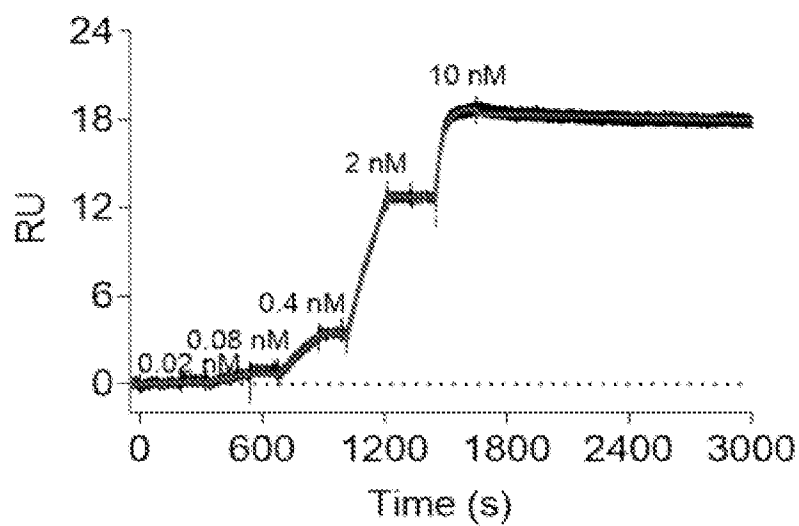


FIG. 1

2/23



Compound	k_a (1/Ms)	k_d (1/s)	K_D (pM)	$t_{1/2}$ (min)	Chi ² (RU ²)	Temp° C
Compound 2	9.40×10^6	1.10×10^{-4}	11.4 (n=2)	116	0.0527	37
Compound 2	3.21×10^6	1.87×10^{-5}	5.8 (n=1)	618	0.012	25
Compound 1	6.13×10^6	1.46×10^{-3}	241 (n=3)	8	0.277	25

FIG. 2

3/23

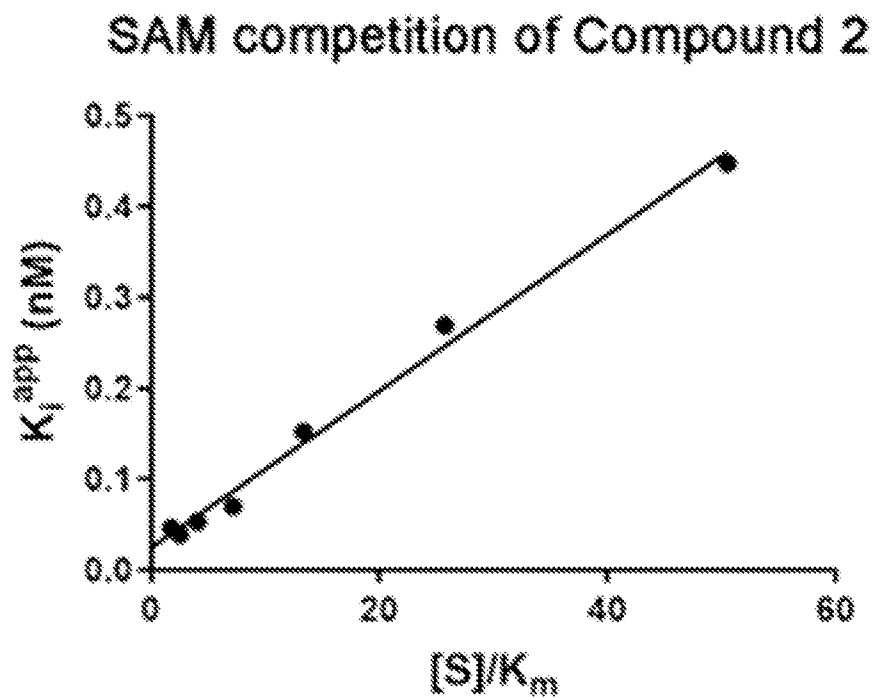


FIG. 3

4/23

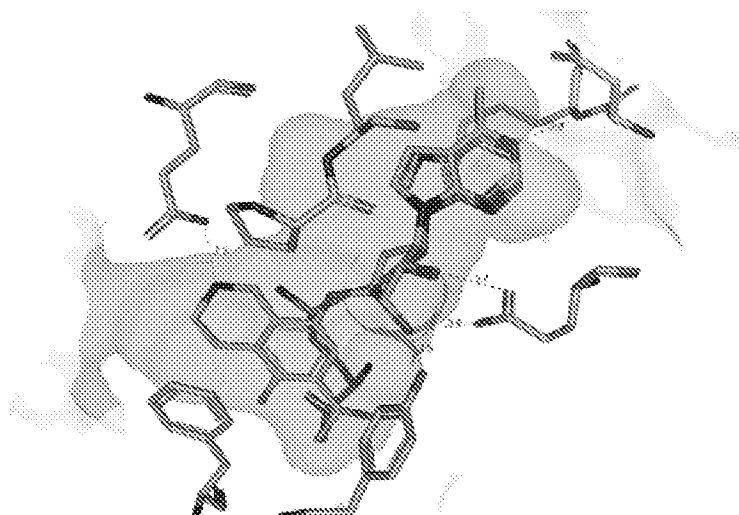


FIG. 4

5/23

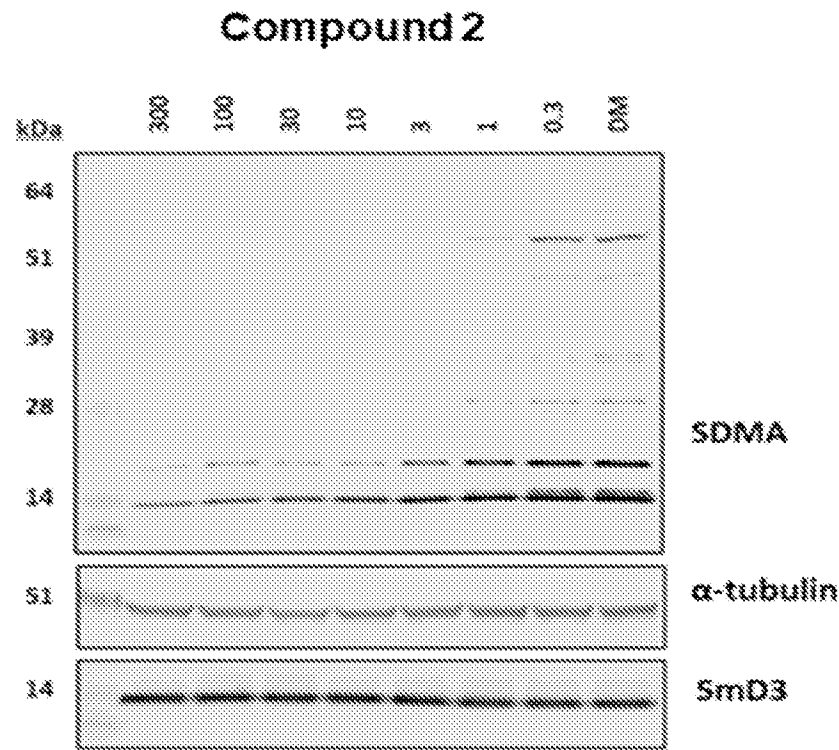


FIG. 5

6/23

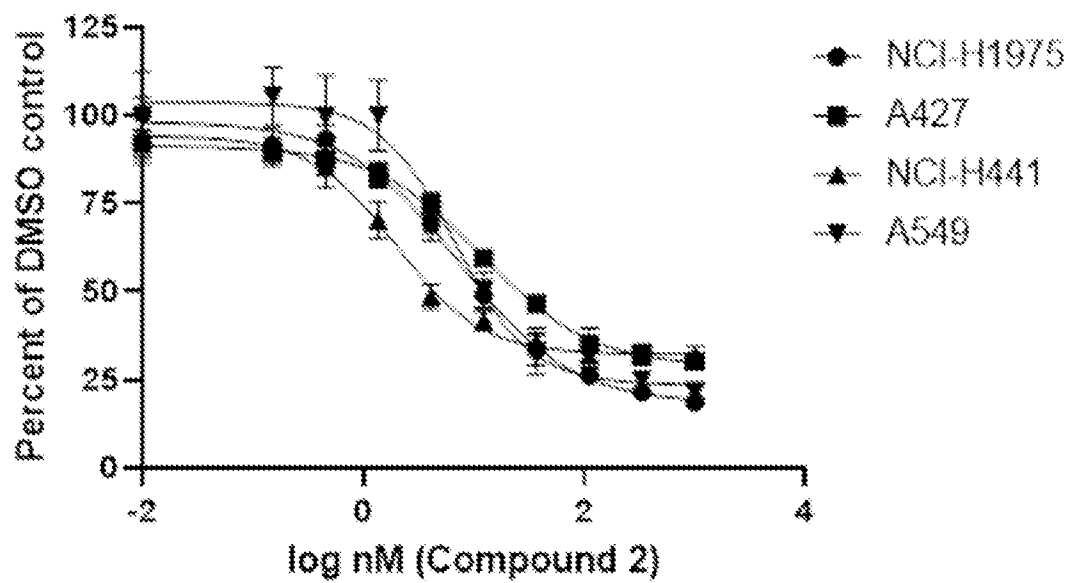


FIG. 6

7/23

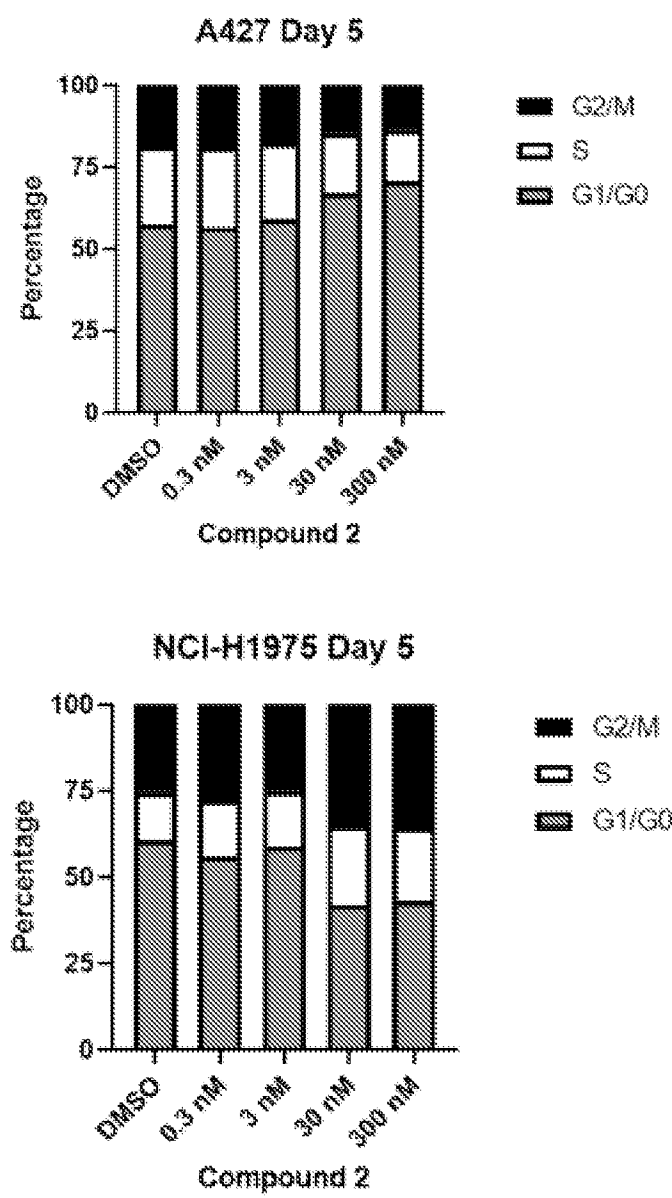


FIG. 7

8/23

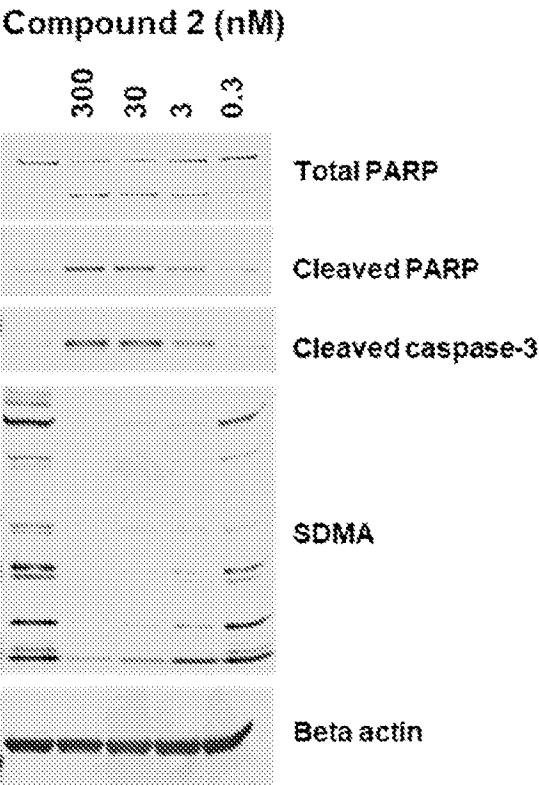


FIG. 8

9/23

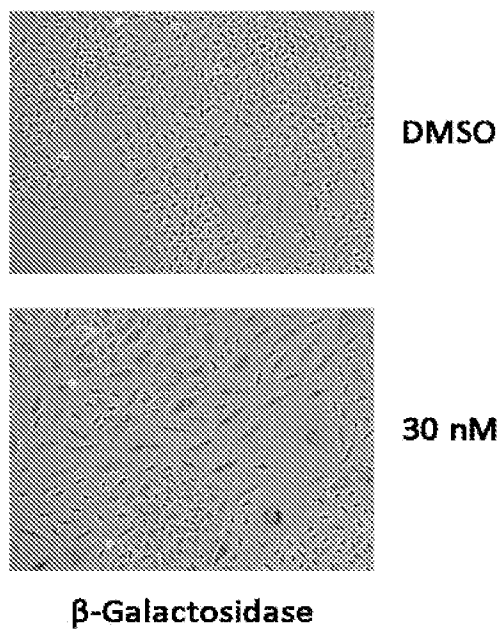


FIG. 9

10/23

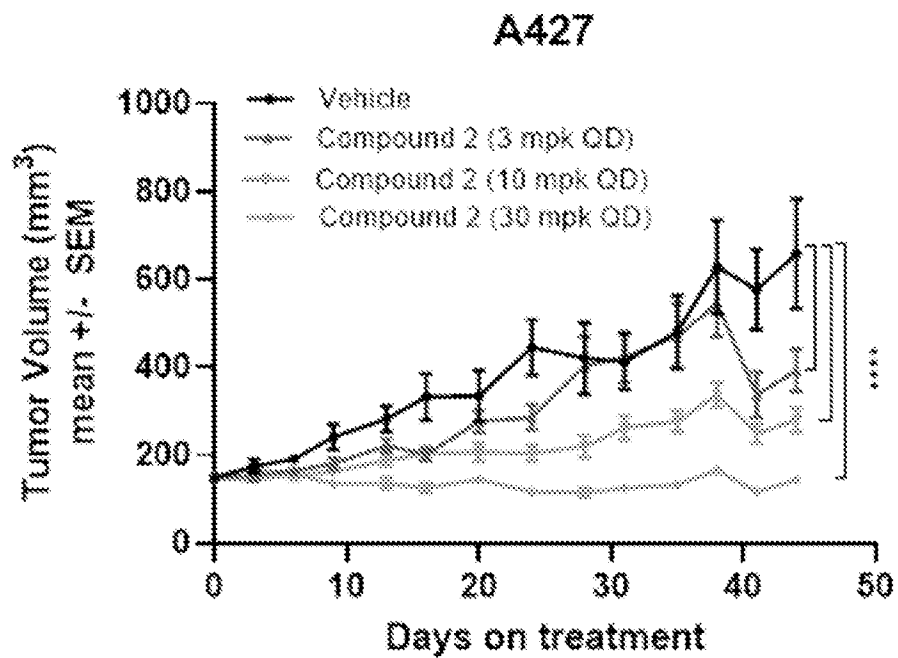


FIG. 10

11/23

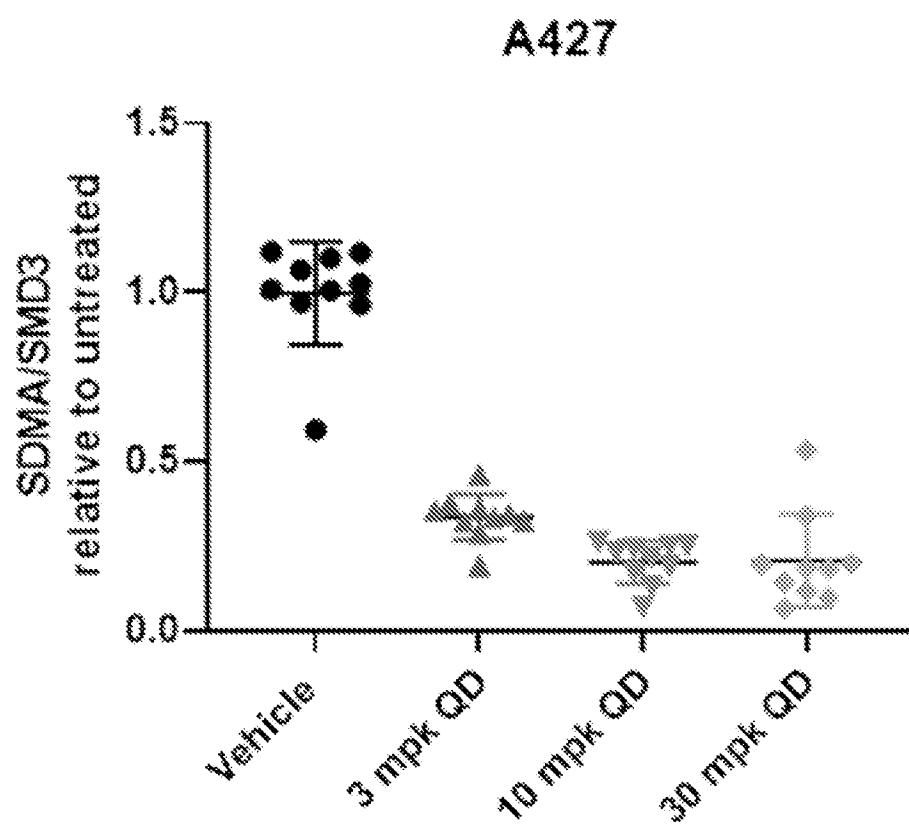


FIG. 11

12/23

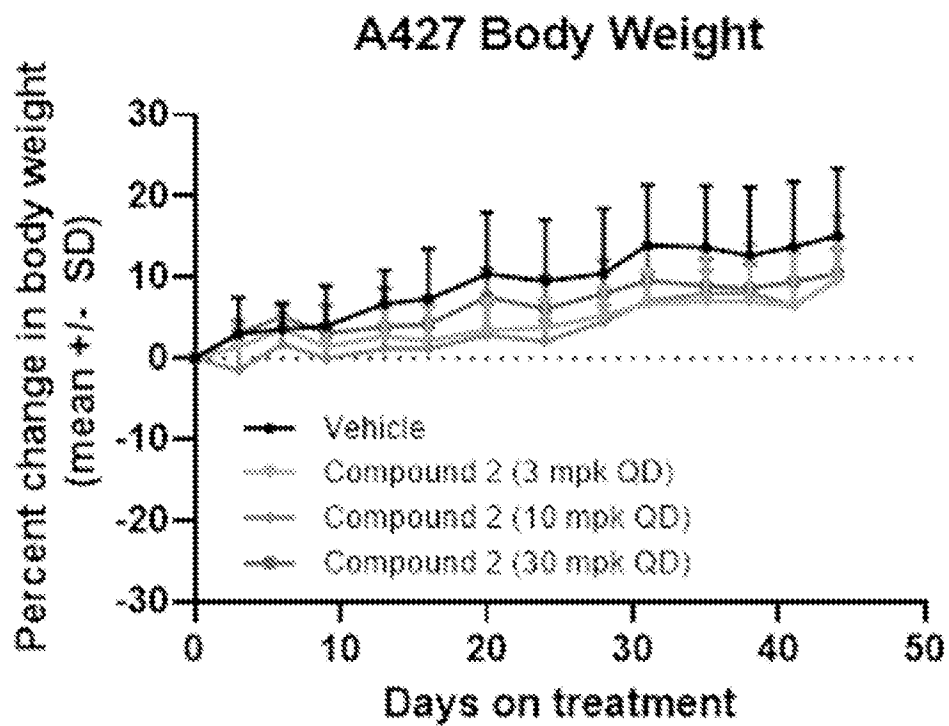


FIG. 12

13/23

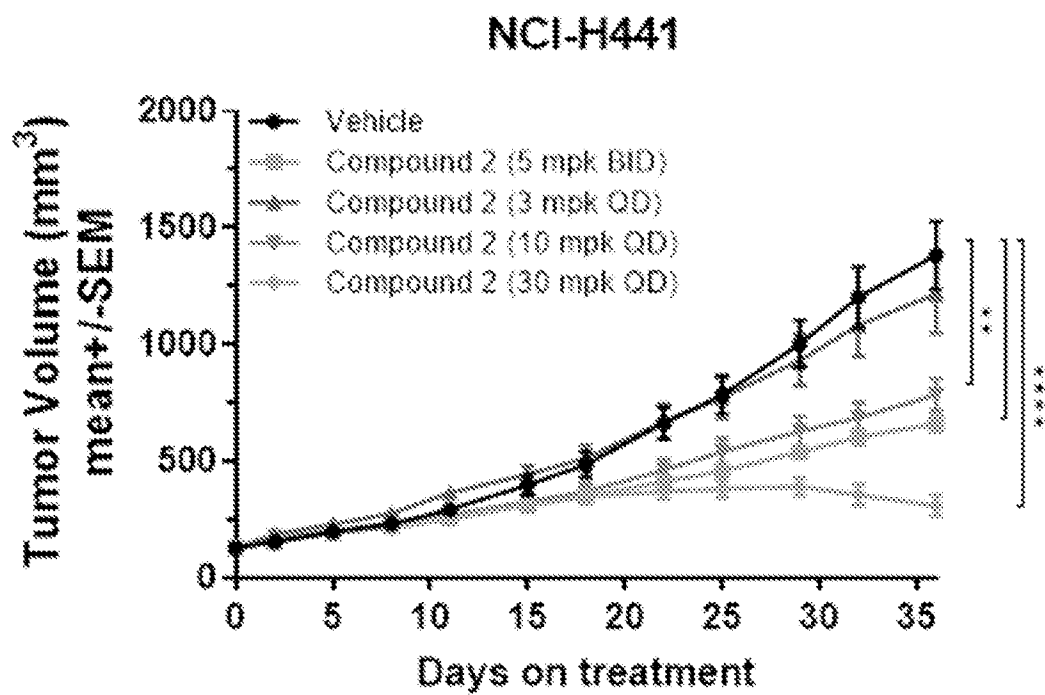


FIG. 13

14/23

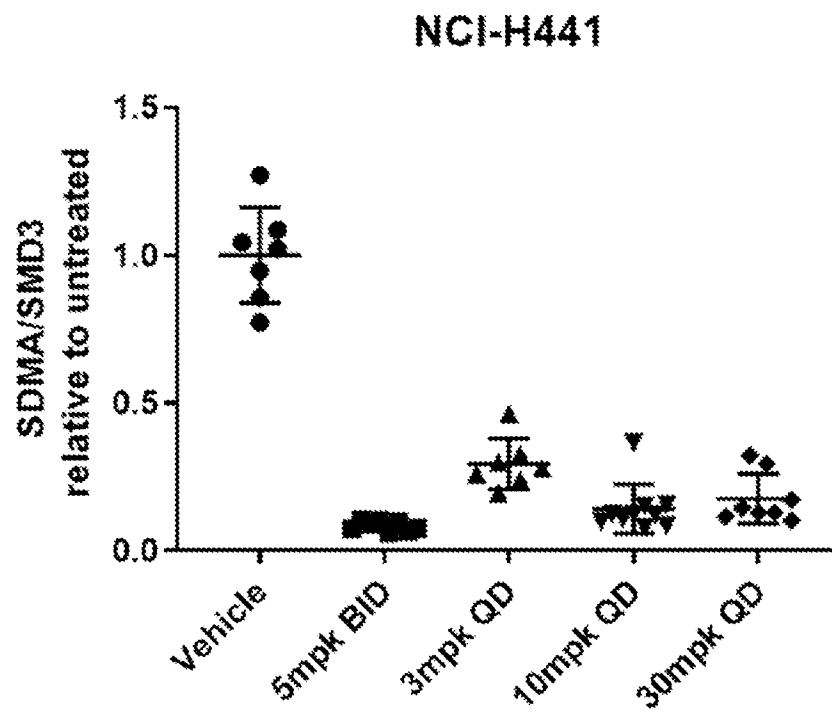


FIG. 14

15/23

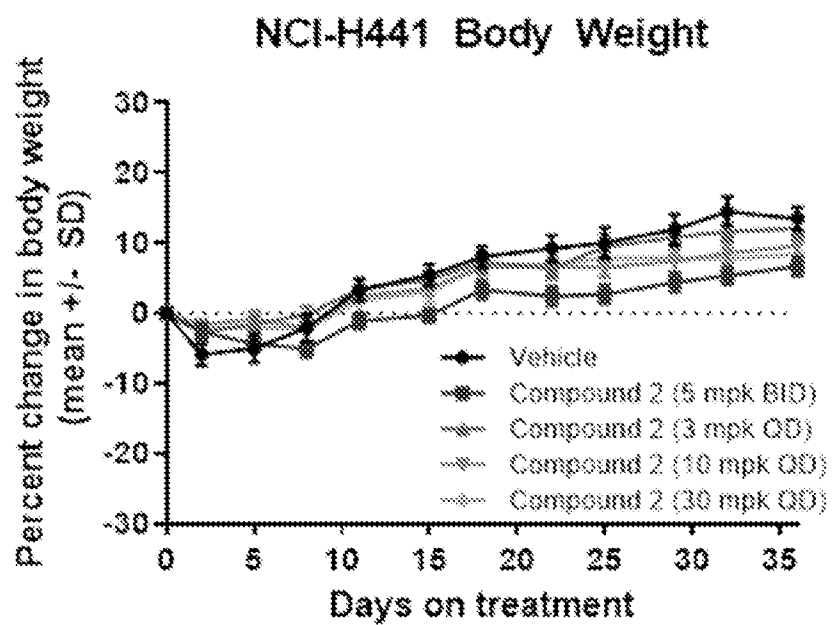


FIG. 15

16/23

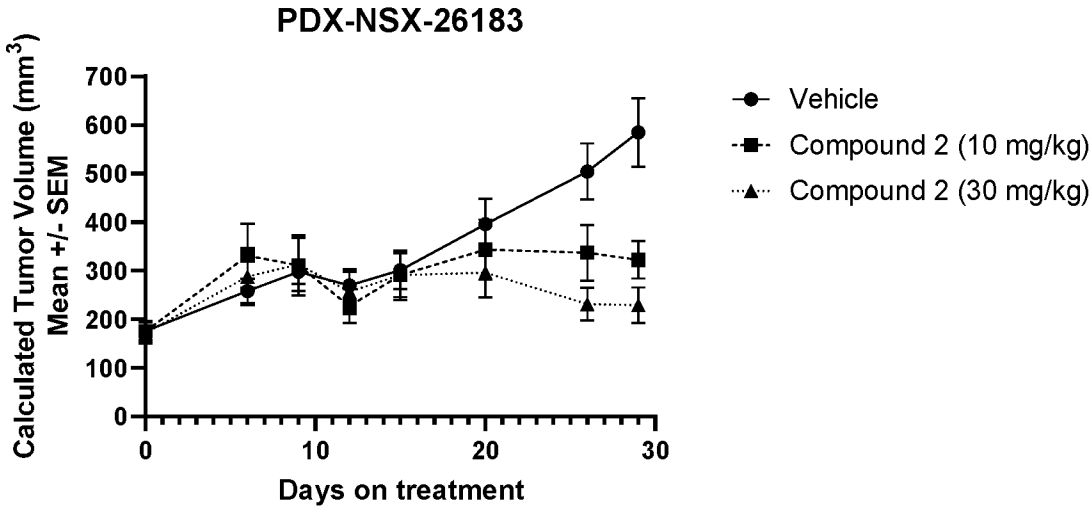


FIG. 16

17/23

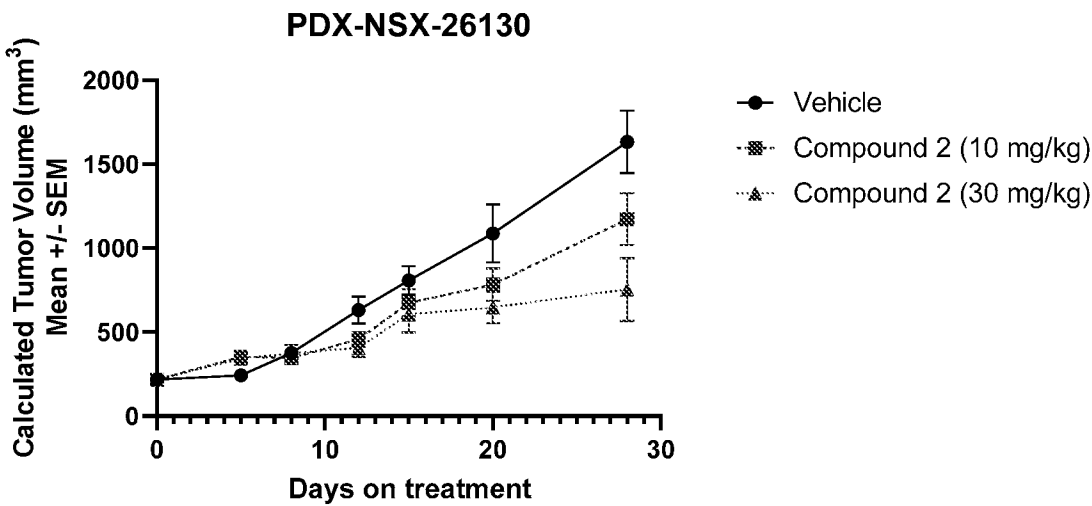


FIG. 17

18/23

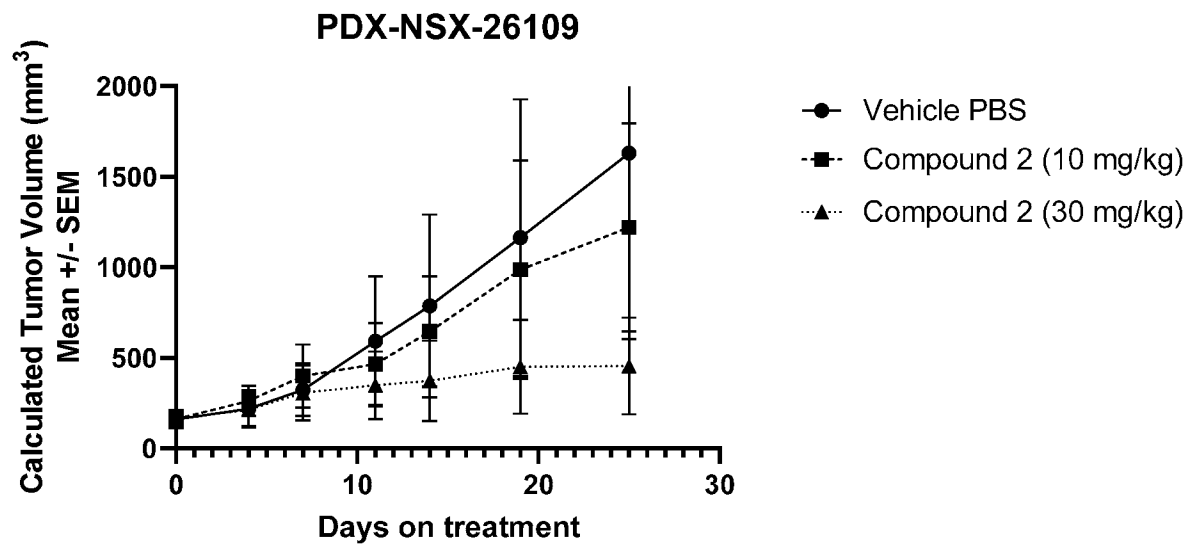


FIG. 18

19/23

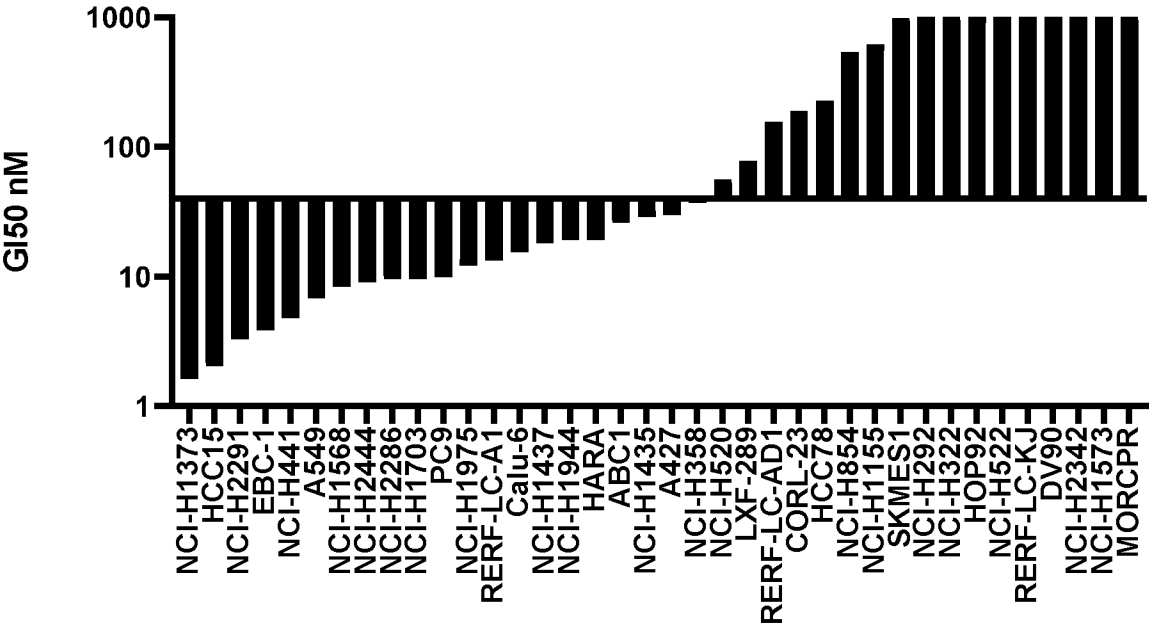


FIG. 19

20/23

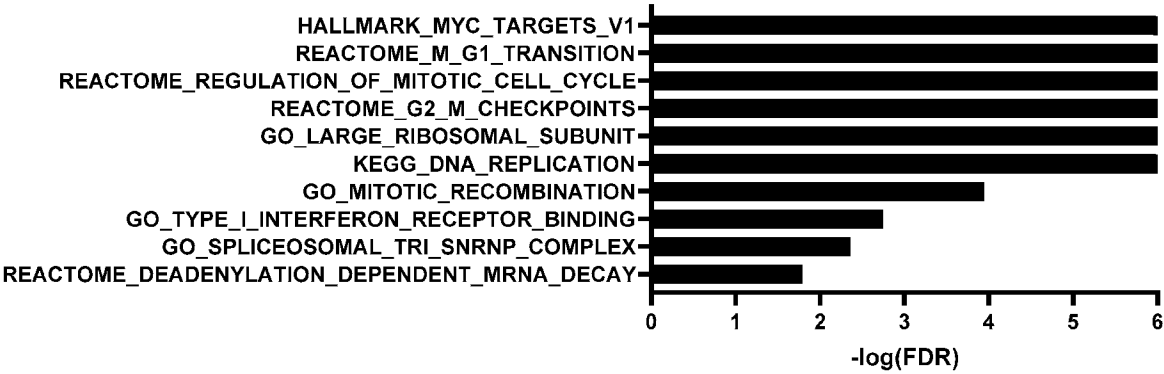


FIG. 20

21/23

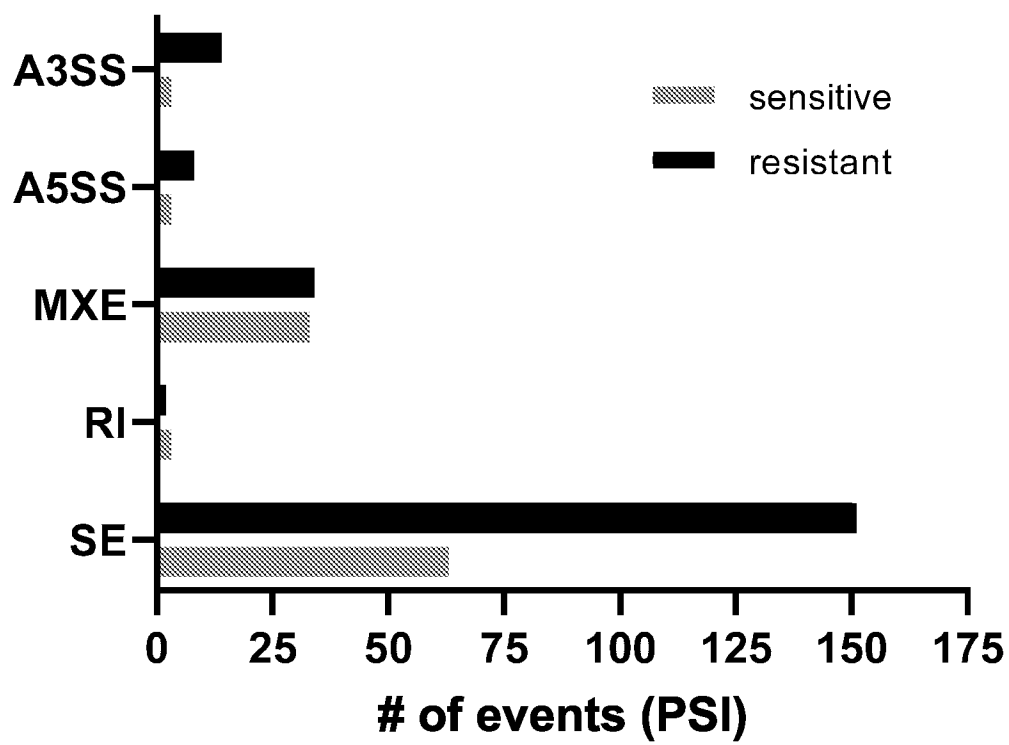


FIG. 21

22/23

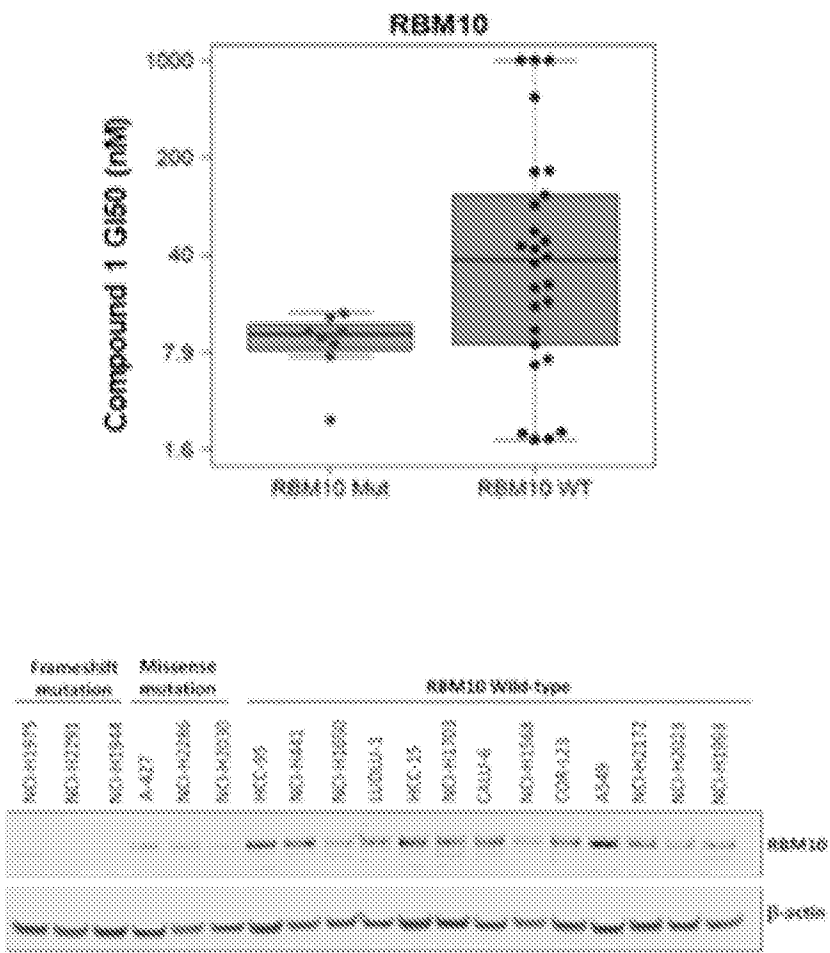


FIG. 22

23/23

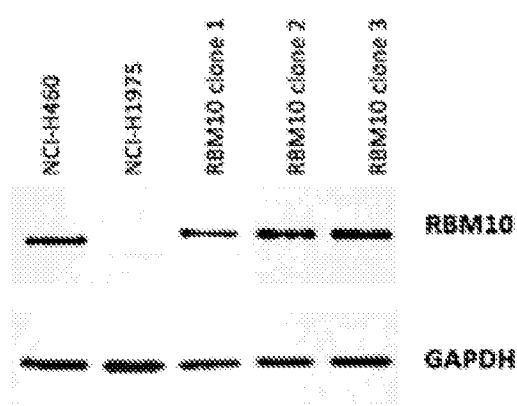
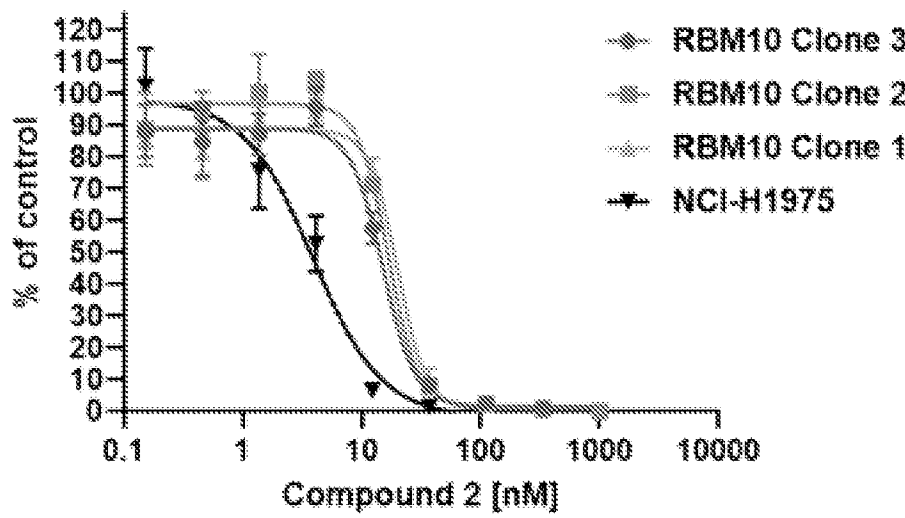


FIG. 23

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2022/050154

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/519 A61K45/06 A61P35/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

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

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

EPO-Internal, BIOSIS, EMBASE, SCISEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2020/384006 A1 (QUINN HILLARY JOY MILLAR [US] ET AL) 10 December 2020 (2020-12-10) paragraph [0080]; claims -----	1, 4, 8-10, 12, 18, 19 1-20
Y		
X	US 2019/111060 A1 (TATLOCK JOHN HOWARD [US] ET AL) 18 April 2019 (2019-04-18) paragraph [0001] - paragraph [0004]; claims ----- -/--	1-20

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

☒ See patent family annex.

* Special categories of cited documents :

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

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search

4 April 2022

Date of mailing of the international search report

14/04/2022

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Fax: (+31-70) 340-3016

Authorized officer

Venturini, Francesca

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2022/050154

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GUCCIONE ERNESTO ET AL: "The regulation, functions and clinical relevance of arginine methylation", NATURE REVIEWS MOLECULAR CELL BIOLOGY, NATURE PUBLISHING GROUP UK, LONDON, vol. 20, no. 10, 26 July 2019 (2019-07-26), pages 642-657, XP036888660, ISSN: 1471-0072, DOI: 10.1038/S41580-019-0155-X [retrieved on 2019-07-26]	1, 12, 19
Y	Type II PRMT inhibitors; table 1	1-20
X	JARROLD JAMES ET AL: "PRMTs and Arginine Methylation: Cancer's Best-Kept Secret?", TRENDS IN MOLECULAR MEDICINE, ELSEVIER CURRENT TRENDS, GB, vol. 25, no. 11, 20 June 2019 (2019-06-20), pages 993-1009, XP085886851, ISSN: 1471-4914, DOI: 10.1016/J.MOLMED.2019.05.007 [retrieved on 2019-06-20]	1, 12, 19
Y	Box 2, Targeting mRNA splicing; figure 1; table 1	1-20
Y	BONDAY ZAHID Q. ET AL: "LLY-283, a Potent and Selective Inhibitor of Arginine Methyltransferase 5, PRMT5, with Antitumor Activity", ACS MEDICINAL CHEMISTRY LETTERS, vol. 9, no. 7, 12 July 2018 (2018-07-12), pages 612-617, XP055906572, US ISSN: 1948-5875, DOI: 10.1021/acsmmedchemlett.8b00014 results, discussion	1-20
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INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	SARAH V. GERHART ET AL: "Activation of the p53-MDM4 regulatory axis defines the anti-tumour response to PRMT5 inhibition through its role in regulating cellular splicing", SCIENTIFIC REPORTS, vol. 8, no. 1, 26 June 2018 (2018-06-26), XP055625791, DOI: 10.1038/s41598-018-28002-y results, discussion -----	1-20
X,P	GUCCIONE ERNESTO ET AL: "Cancer synthetic vulnerabilities to protein arginine methyltransferase inhibitors", CURRENT OPINION IN PHARMACOLOGY, ELSEVIER SCIENCE PUBLISHERS, NL, vol. 59, 27 May 2021 (2021-05-27), pages 33-42, XP086718335, ISSN: 1471-4892, DOI: 10.1016/J.COPH.2021.04.004 [retrieved on 2021-05-27] tumors with point mutations in splicing factors; table 1 -----	1-20
X,P	JENSEN-PERGAKES KRISTEN ET AL: "SAM-Competitive PRMT5 Inhibitor PF-06939999 Demonstrates Antitumor Activity in Splicing Dysregulated NSCLC with Decreased Liability of Drug Resistance", MOLECULAR CANCER THERAPEUTICS, vol. 21, no. 1, 4 November 2021 (2021-11-04), pages 3-15, XP055908169, US ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-21-0620 results, discussion ----- -/--	1-20

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2022/050154

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	Jensen-Pergakes K. ET AL: "Abstract 1160: SAM competitive PRMT5 inhibitor PF06939999 demonstrates antitumor activity in splicing dysregulated NSCLC with decreased liability of drug resistance Cancer Research American Association for Cancer Research", , 1 July 2021 (2021-07-01), XP55908162, Retrieved from the Internet: URL: https://aacrjournals.org/cancerres/article/81/13_Supplement/1160/667047/Abstract-1160-SAM-competitive-PRMT5-inhibitor [retrieved on 2022-04-01] abstract -----	1-20

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2022/050154

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
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 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2022/050154

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