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(54) **Title:** TREATMENT OF CANCER WITH KDM4 INHIBITORS

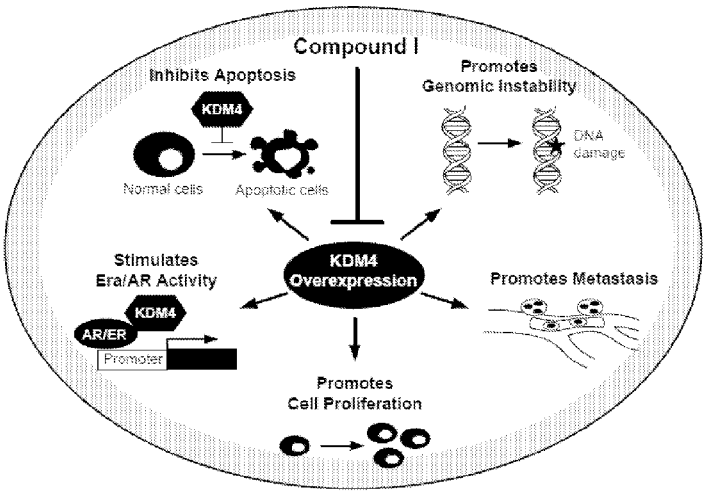


FIG. 1

(57) **Abstract:** Provided herein are compositions and methods for the treatment of a cancer. Said compositions comprise a KDM4 inhibitor.

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TREATMENT OF CANCER WITH KDM4 INHIBITORS

CROSS-REFERENCE TO RELATED APPLICATION

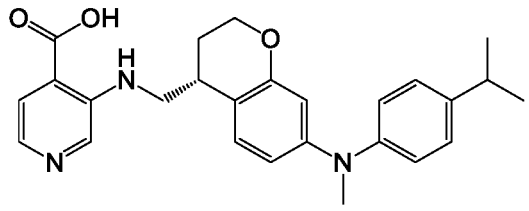
[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/173,219, filed on April 9, 2021; U.S. Provisional Patent Application No. 63/252,542, filed on October 5, 2021; and U.S. Provisional Patent Application No. 63/297,960, filed on January 10, 2022, all are hereby incorporated by reference in their entirety.

BRIEF SUMMARY

[0002] Provided herein are compositions and methods for the treatment of cancer. The types of cancer suitable for the methods disclosed herein include, but are not limited to, colorectal cancer, triple negative breast cancer, gastric adenocarcinoma, and diffuse large B-cell non-Hodgkin's lymphoma.

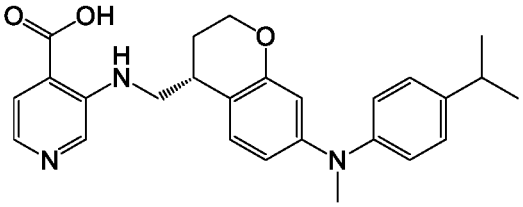
[0003] The compositions useful for the methods of treating cancer disclosed herein comprise heterocyclic KDM4 inhibitors described herein.

[0004] One embodiment provides a method of treating a cancer in a cancer patient in need thereof, comprising administering to the individual a compound, or pharmaceutically acceptable salt or solvate thereof, having the structure:

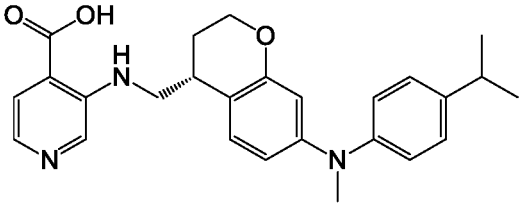


. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from colorectal cancer, esophageal cancer, triple negative breast cancer, gastric cancer, lymphoma, gastric adenocarcinoma, diffuse large B-cell non-Hodgkin's lymphoma, acute T-cell leukemia, esophageal squamous cell carcinoma, multiple myeloma, acute myeloid leukemia, colorectal adenocarcinoma, colorectal carcinoma, pancreatic cancer, pancreatic carcinoma, breast carcinoma, or T-cell acute lymphoblastic leukemia.

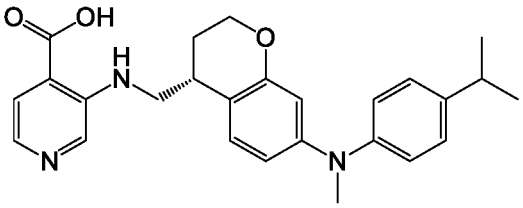
[0005] Another embodiment provides a method of reducing tumorigenic cell population in cancer patient in need thereof, comprising administering to the individual a compound, or pharmaceutically acceptable salt or solvate thereof, having the structure:



[0006] Another embodiment provides a method of reducing tumor initiating cell frequency in a cancer patient in need thereof, comprising administering to the individual a compound, or pharmaceutically acceptable salt or solvate thereof, having the structure:



[0007] Another embodiment provides a method of inhibiting cancer stem cells in a cancer patient in need thereof, comprising administering to the individual a compound, or pharmaceutically acceptable salt or solvate thereof, having the structure:



BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Fig. 1 illustrates the biochemical activities of Compound 1, a potent PAN KDM4 inhibitor.

[0009] Fig. 2 illustrates the reversible and competitive inhibition of H3K9me3 demethylation. Inhibition of H3K3me3 demethylation was measured at various Compound 1 concentrations in the presence of α-KG using time-resolved fluorescence-based (TR-FRET) LANCE® detection.

[0010] Fig. 3 illustrates that Compound 1 induces cell cycle arrest in S-phase. Compound 1 increased proportion of cells in S-phase in a dose-dependent manner. Cell cycle analysis was performed with MDA-MB-231 cells and using MultiCycle AV DNA analysis software.

[0011] Fig. 4 demonstrates that Compound 1 induces apoptosis in human cancer cell lines.

[0012] Fig. 5 demonstrates that Compound 1 exhibits potent anti-proliferative activity across a large panel of cancer cell lines. Evaluation of compound 1 in a panel of PDX/organoid models confirms compound 1 sensitivity in gastric cancers and shows sensitivity to MSI CRC vs. MSS CRC.

[0013] Fig. 6 illustrates the dose-dependent inhibition of histone demethylase by Compound 1. Left Panel: Representative immunoblot showing change in staining intensity for actin (red bands) and H3K36me3 (green bands) in response to Compound 1 at various concentrations. Right Panel: Normalized ratio of band intensities for H3K36me3 and actin versus the concentration of Compound 1 (μM). (mean IC50 ±SD value – 0.085 ±0.004 μM).

[0014] Fig. 7 illustrates the reduction of tumorigenic cells upon treatment of tumors with Compound 1. Compound 1 reduced tumor initiating cell (TIC) frequency by 4.4-fold.

[0015] Fig. 8 illustrates the in vivo efficacy of Compound 1 in multiple cancer models.

[0016] Fig. 9 illustrates inhibition of cell proliferation in the SU60 organoid model.

[0017] Fig. 10 *In vivo* efficacy of Compound 1 in the SU60 patient-derived colorectal cancer xenograft model.

[0018] Fig. 11 *In vivo* efficacy in study no. 1 of Compound 1 in the KYSE-150 human esophageal cancer xenograft model.

[0019] Fig. 12 *In vivo* efficacy in study no. 2 of Compound 1 in the KYSE-150 human esophageal cancer xenograft model.

[0020] Fig. 13 *In vivo* efficacy of Compound 1 in the COH70 patient-derived triple negative breast cancer xenograft model.

[0021] Fig. 14 *In vivo* Efficacy of Compound 1 in the GXA-3036 gastric adenocarcinoma patient-derived xenograft model.

[0022] Fig. 15 *In vivo* Efficacy of Compound 1 in the OCI-LY19 human diffuse large B-cell non-Hodgkin's lymphoma xenograft model.

[0023] Fig. 16 illustrates flow cytometry analysis of tumorigenic cell population after Compound 1 treatment.

[0024] Fig. 17 illustrates the results of the in vivo tumorigenicity functional assay after Compound 1 treatment.

[0025] Fig. 18 illustrates a heatmap of the mutation status of genes in various pathways in different colorectal cancer cell lines. The respective Microsatellite instability (MSI-H), CpG island methylator phenotype (CIMP), and MLH1 methylation status as well as the sensitivity of these cell lines are to Compound 1 are also shown.

[0026] Fig. 19 illustrates a heatmap showing the MSI-H status and respective MMR path gene mutations of various colorectal cancer cell lines studied.

[0027] Fig. 20 illustrates that genes PNUTS (PPP1R10), ANK1, IBA57, SOWAHD, MF12-AS1, and CECR1 exhibited dose-dependent changes following treatment with Compound 1 *in vivo*.

[0028] Fig. 21 illustrates that ChIP-seq analysis in MDA-MB-231 TNBC cells shows KDM4 occupancy at the PNUTS (PPP1R10) promoter.

[0029] Fig. 22 illustrates the heatmap representation of single cells gene expression analysis (RT-qPCR) from SU60 xenograft tumors treated with vehicle control or Compound 1. A) Heatmap representation of single cells gene expression analysis (RT-qPCR) from SU60 xenograft tumors treated with vehicle control or Compound 1. Gene expression levels are colored-coded with red for high expression, green low expression and gray not expressed. Three clusters of cells were identified based on shared patterns of gene expression using a gene-set of immature and mature cell markers. On the far right of the heatmap a red/blue ribbon displays the distribution of 335 vehicle-treated (blue) and 332 compound 1-treated (red) single cells. The top dendrogram clusters genes with similar expression pattern across all cells. Gene expression (in Ct values) was mean-centered at 0 and divided by 2.5 time the standard deviation. B) This graph represents the total number of vehicle-treated and compound 1 treated cells within each cluster. P-values (Fisher's exact test) are represented on top of the bars.

INCORPORATION BY REFERENCE

[0030] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference for the specific purposes identified herein.

DETAILED DESCRIPTION

Certain Terminology

[0031] As used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an agent" includes a plurality of such agents, and reference to "the cell" includes reference to one or more cells (or to a plurality of cells) and equivalents thereof known to those skilled in the art, and so forth. When ranges are used herein for physical properties, such as molecular weight, or chemical properties, such as chemical formulae, all combinations and sub-combinations of ranges and specific embodiments therein are intended to be included. The term "about" when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range, in some instances, will vary between 1% and 15% of the stated number or numerical range. The term "comprising" (and related terms such as "comprise" or "comprises" or "having" or "including") is not intended to exclude that in other certain embodiments, for example, an embodiment of any composition of

matter, composition, method, or process, or the like, described herein, "consist of" or "consist essentially of" the described features.

[0032] As used in the specification and appended claims, unless specified to the contrary, the following terms have the meaning indicated below.

[0033] The compounds disclosed herein, in some embodiments, are used in different enriched isotopic forms, e.g., enriched in the content of ^{2}H, ^{3}H, ^{11}C, ^{13}C and/or ^{14}C. In one particular embodiment, the compound is deuterated in at least one position. Such deuterated forms can be made by the procedure described in U.S. Patent Nos. 5,846,514 and 6,334,997. As described in U.S. Patent Nos. 5,846,514 and 6,334,997, deuteration can improve the metabolic stability and or efficacy, thus increasing the duration of action of drugs.

[0034] Unless otherwise stated, structures depicted herein are intended to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by ^{13}C- or ^{14}C-enriched carbon are within the scope of the present disclosure.

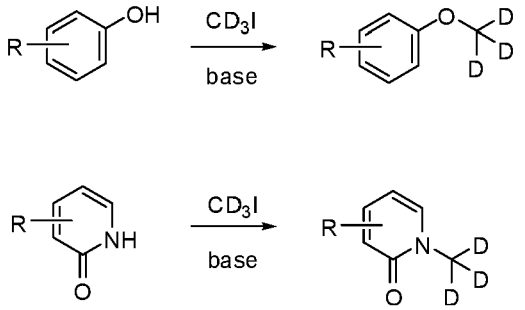
[0035] The compounds of the present disclosure optionally contain unnatural proportions of atomic isotopes at one or more atoms that constitute such compounds. For example, the compounds may be labeled with isotopes, such as for example, deuterium (^{2}H), tritium (^{3}H), iodine-125 (^{125}I) or carbon-14 (^{14}C). Isotopic substitution with ^{2}H, ^{11}C, ^{13}C, ^{14}C, ^{15}C, ^{12}N, ^{13}N, ^{15}N, ^{16}N, ^{16}O, ^{17}O, ^{14}F, ^{15}F, ^{16}F, ^{17}F, ^{18}F, ^{33}S, ^{34}S, ^{35}S, ^{36}S, ^{35}Cl, ^{37}Cl, ^{79}Br, ^{81}Br, ^{125}I are all contemplated. In some embodiments, isotopic substitution with ^{18}F is contemplated. All isotopic variations of the compounds of the present invention, whether radioactive or not, are encompassed within the scope of the present invention.

[0036] In certain embodiments, the compounds disclosed herein have some or all of the ^{1}H atoms replaced with ^{2}H atoms. The methods of synthesis for deuterium-containing compounds are known in the art and include, by way of non-limiting example only, the following synthetic methods.

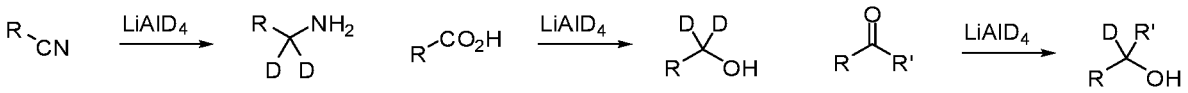
[0037] Deuterium substituted compounds are synthesized using various methods such as described in: Dean, Dennis C.; Editor. Recent Advances in the Synthesis and Applications of Radiolabeled Compounds for Drug Discovery and Development. [Curr., Pharm. Des., 2000; 6(10)] 2000, 110 pp; George W.; Varma, Rajender S. The Synthesis of Radiolabeled Compounds via Organometallic Intermediates, Tetrahedron, 1989, 45(21), 6601-21; and Evans, E. Anthony. Synthesis of radiolabeled compounds, J. Radioanal. Chem., 1981, 64(1-2), 9-32.

[0038] Deuterated starting materials are readily available and are subjected to the synthetic methods described herein to provide for the synthesis of deuterium-containing compounds. Large numbers of deuterium-containing reagents and building blocks are available commercially from chemical vendors, such as Aldrich Chemical Co.

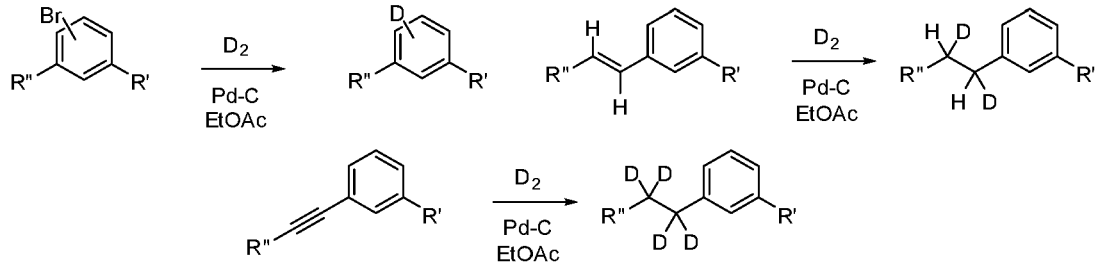
[0039] Deuterium-transfer reagents suitable for use in nucleophilic substitution reactions, such as iodomethane-d_3 (CD_3I), are readily available and may be employed to transfer a deuterium-substituted carbon atom under nucleophilic substitution reaction conditions to the reaction substrate. The use of CD_3I is illustrated, by way of example only, in the reaction schemes below.



[0040] Deuterium-transfer reagents, such as lithium aluminum deuteride ($LiAlD_4$), are employed to transfer deuterium under reducing conditions to the reaction substrate. The use of $LiAlD_4$ is illustrated, by way of example only, in the reaction schemes below.



[0041] Deuterium gas and palladium catalyst are employed to reduce unsaturated carbon-carbon linkages and to perform a reductive substitution of aryl carbon-halogen bonds as illustrated, by way of example only, in the reaction schemes below.



[0042] In one embodiment, the compounds disclosed herein contain one deuterium atom. In another embodiment, the compounds disclosed herein contain two deuterium atoms. In another embodiment, the compounds disclosed herein contain three deuterium atoms. In another embodiment, the compounds disclosed herein contain four deuterium atoms. In another embodiment, the compounds disclosed herein contain five deuterium atoms. In another

embodiment, the compounds disclosed herein contain six deuterium atoms. In another embodiment, the compounds disclosed herein contain more than six deuterium atoms. In another embodiment, the compound disclosed herein is fully substituted with deuterium atoms and contains no non-exchangeable ^{1}H hydrogen atoms. In one embodiment, the level of deuterium incorporation is determined by synthetic methods in which a deuterated synthetic building block is used as a starting material.

[0043] "Pharmaceutically acceptable salt" includes both acid and base addition salts. A pharmaceutically acceptable salt of heterocyclic KDM4 inhibitor described herein is intended to encompass any and all pharmaceutically suitable salt forms. Preferred pharmaceutically acceptable salts of the compounds described herein are pharmaceutically acceptable acid addition salts and pharmaceutically acceptable base addition salts.

[0044] "Pharmaceutically acceptable acid addition salt" refers to those salts which retain the biological effectiveness and properties of the free bases, which are not biologically or otherwise undesirable, and which are formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, hydroiodic acid, hydrofluoric acid, phosphorous acid, and the like. Also included are salts that are formed with organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, alkanedioic acids, aromatic acids, aliphatic and. aromatic sulfonic acids, etc. and include, for example, acetic acid, trifluoroacetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like. Exemplary salts thus include sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, nitrates, phosphates, monohydrogenphosphates, dihydrogenphosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, trifluoroacetates, propionates, caprylates, isobutyrates, oxalates, malonates, succinate suberates, sebacates, fumarates, maleates, mandelates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, phthalates, benzenesulfonates, toluenesulfonates, phenylacetates, citrates, lactates, malates, tartrates, methanesulfonates, and the like. Also contemplated are salts of amino acids, such as arginates, gluconates, and galacturonates (see, for example, Berge S.M. et al., "Pharmaceutical Salts," *Journal of Pharmaceutical Science*, 66:1-19 (1997)). Acid addition salts of basic compounds are, in some embodiments, prepared by contacting the free base forms with a sufficient amount of the desired acid to produce the salt according to methods and techniques with which a skilled artisan is familiar.

[0045] "Pharmaceutically acceptable base addition salt" refers to those salts that retain the biological effectiveness and properties of the free acids, which are not biologically or otherwise

undesirable. These salts are prepared from addition of an inorganic base or an organic base to the free acid. Pharmaceutically acceptable base addition salts are, in some embodiments, formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Salts derived from inorganic bases include, but are not limited to, sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, for example, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, diethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, *N,N*-dibenzylethylenediamine, chloroprocaine, hydrabamine, choline, betaine, ethylenediamine, ethylenedianiline, *N*-methylglucamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. See Berge et al., supra.

[0046] "Pharmaceutically acceptable solvate" refers to a composition of matter that is the solvent addition form. In some embodiments, solvates contain either stoichiometric or non-stoichiometric amounts of a solvent, and are formed during the process of making with pharmaceutically acceptable solvents such as water, ethanol, and the like. Hydrates are formed when the solvent is water, or alcoholates are formed when the solvent is alcohol. Solvates of compounds described herein are conveniently prepared or formed during the processes described herein. The compounds provided herein optionally exist in either unsolvated as well as solvated forms.

[0047] The term "subject" or "patient" encompasses mammals. Examples of mammals include, but are not limited to, any member of the Mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. In one aspect, the mammal is a human.

[0048] As used herein, "treatment" or "treating," or "palliating" or "ameliorating" are used interchangeably. These terms refer to an approach for obtaining beneficial or desired results including but not limited to therapeutic benefit and/or a prophylactic benefit. By "therapeutic benefit" is meant eradication or amelioration of the underlying disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the patient, notwithstanding that the patient is still afflicted with the underlying disorder. For prophylactic benefit, the compositions are, in some embodiments, administered to

a patient at risk of developing a particular disease, or to a patient reporting one or more of the physiological symptoms of a disease, even though a diagnosis of this disease has not been made. The term "treating", as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. In some embodiments, the tern “treating” includes slowing or delaying the progression of the disease or disorder to which the term is applied. Additionally, in some embodiments, the term “treating” is applied to one or more of the complications resulting from the disease or disorder to which the term is applied. The term "treatment", as used herein, unless otherwise indicated, refers to the act of treating as "treating" is defined immediately above.

[0049] The term "tumor," or “cancer” as used herein, and unless otherwise specified, refers to a neoplastic cell growth, and includes pre-cancerous and cancerous cells and tissues. Tumors usually present as a lesion or lump. As used herein, “treating” a tumor means that one or more symptoms of the disease, such as the tumor itself, vascularization of the tumor, or other parameters by which the disease is characterized, are reduced, ameliorated, inhibited, placed in a state of remission, or maintained in a state of remission. “Treating” a tumor also means that one or more hallmarks of the tumor may be eliminated, reduced or prevented by the treatment. Non-limiting examples of such hallmarks include uncontrolled degradation of the basement membrane and proximal extracellular matrix, migration, division, and organization of the endothelial cells into new functioning capillaries, and the persistence of such functioning capillaries.

[0050] The term “refractory” or “refractory to therapy” indicates that the patients have never responded to therapy.

[0051] The term “relapsed” or “relapsed after therapy” indicates that patients, after initially responding to therapy, have progressive disease due to acquired resistance and/or intolerance.

[0052] The term “resistance to therapy” or “acquired resistance to therapy” indicates the patients, after initially responding to therapy, have progressive disease due to clinical or molecular resistance to the therapy. The acquired resistance can result from emergence of resistant mutations in the molecular target of the therapy, or in the development of physiological functions such as efflux pumps.

[0053] The phrase "therapeutically effective amount", as used herein, refers to that amount of drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal, or human that is being sought by a researcher, veterinarian, medical doctor or other.

[0054] The term “tumorigenic” indicates a cell that is capable of forming or tending to form tumors.

[0055] The term “tumorigenic cell population” indicates a population of cells that have neoplastic properties and are capable of forming tumors.

[0056] The term “tumor initiating cell” or “cancer stem cell” refers to cell(s) that produce the various differentiated progeny that make up the bulk of the rapidly dividing tumor.

[0057] Other aspects, advantages, and features of the invention will become apparent from the detailed description below.

Histone Demethylase

[0058] Chromatin is the complex of DNA and protein that makes up chromosomes. Histones are the major protein component of chromatin, acting as spools around which DNA winds. Changes in chromatin structure are affected by covalent modifications of histone proteins and by non-histone binding proteins. Several classes of enzymes are known which can covalently modify histones at various sites.

[0059] Proteins can be post-translationally modified by methylation on amino groups of lysines and guanidino groups of arginines or carboxymethylated on aspartate, glutamate, or on the C-terminus of the protein. Post-translational protein methylation has been implicated in a variety of cellular processes such as RNA processing, receptor mediated signaling, and cellular differentiation. Post-translational protein methylation is widely known to occur on histones, such reactions known to be catalyzed by histone methyltransferases, which transfer methyl groups from S-adenyosyl methionine (SAM) to histones. Histone methylation is known to participate in a diverse range of biological processes including heterochromatin formation, X-chromosome inactivation, and transcriptional regulation (Lachner et al., (2003) J. Cell Sci. 116:2117-2124; Margueron et al., (2005) Curr. Opin. Genet. Dev. 15:163-176).

[0060] Unlike acetylation, which generally correlates with transcriptional activation, whether histone methylation leads to transcription activation or repression depends on the particular site of methylation and the degree of methylation (e.g., whether a particular histone lysine residue is mono-, di-, or tri-methylated). However, generally, methylation on H3K9, H3K27 and H4K20 is linked to gene silencing, while methylation on H3K4, H3K36, and H3K79 is generally associated with active gene expression. In addition, tri- and di-methylation of H3K4 generally marks the transcriptional start sites of actively transcribed genes, whereas mono-methylation of H3K4 is associated with enhancer sequences.

[0061] A "demethylase" or "protein demethylase," as referred to herein, refers to an enzyme that removes at least one methyl group from an amino acid side chain. Some demethylases act on

histones, e.g., act as a histone H3 or H4 demethylase. For example, an H3 demethylase may demethylate one or more of H3K4, H3K9, H3K27, H3K36 and/or H3K79. Alternately, an H4 demethylase may demethylate histone H4K20. Demethylases are known which can demethylate either a mono-, di- and/or a tri-methylated substrate. Further, histone demethylases can act on a methylated core histone substrate, a mononucleosome substrate, a dinucleosome substrate and/or an oligonucleosome substrate, peptide substrate and/or chromatin (e.g., in a cell-based assay).

[0062] The first lysine demethylase discovered was lysine specific demethylase 1 (LSD1/KDM1), which demethylates both mono- and di-methylated H3K4 or H3K9, using flavin as a cofactor. A second class of Jumonji C (JmjC) domain containing histone demethylases were predicted, and confirmed when a H3K36 demethylase was found using a formaldehyde release assay, which was named JmjC domain containing histone demethylase 1 (JHDM1/KDM2A).

[0063] More JmjC domain-containing proteins were subsequently identified and they can be phylogenetically clustered into seven subfamilies: JHDM1, JHDM2, JHDM3, JMJD2, JARID, PHF2/PHF8, UTX/UTY, and JmjC domain only.

JMJD2 Family

[0064] The JMJD2 family of proteins are a family of histone-demethylases known to demethylate tri- and di-methylated H3-K9, and were the first identified histone tri-methyl demethylases. In particular, ectopic expression of JMJD2 family members was found to dramatically decrease levels of tri-and di-methylated H3-K9, while increasing levels of mono-methylated H3-K9, which delocalized Heterochromatin Protein 1 (HPl) and reduced overall levels of heterochromatin in vivo. Members of the JMJD2 subfamily of jumonji proteins include JMJD2C and its homologues JMJD2A, JMJD2B, JMJD2D and JMJD2E. Common structural features found in the JMJD2 subfamily of Jumonji proteins include the JmjN, JmjC, PHD and Tdr sequences.

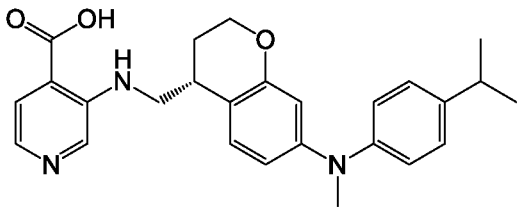
[0065] JMJD2C, also known as GASC1 and KDM4C, is known to demethylate tri-methylated H3K9 and H3K36. Histone demethylation by JMJD2C occurs via a hydroxylation reaction dependent on iron and α-ketoglutarate., wherein oxidative decarboxylation of α-ketoglutarate by JMJD2C produces carbon dioxide, succinate, and ferryl and ferryl subsequently hydroxylates a methyl group of lysine H3K9, releasing formaldehyde. JMJD2C is known to modulate regulation of adipogenesis by the nuclear receptor PPARγ and is known to be involved in regulation of self-renewal in embryonic stem cells.

[0066] KDM4 histone lysine demethylase is an epigenetic regulator and key oncogenic driver across multiple tumor types. The KDM4 family consists of four main isoforms (KDM4A, B, C,

D); all have been implicated in epigenetic dysregulation in various cancers (Zack et al., 2013). KDM4 controls transition between transcriptionally silent and active chromatin states via removal of methyl marks on histone H3K9 and histone H3K36. KDM4 is also necessary for self-renewal of embryonic stem cells and the generation of induced pluripotent stem cells (Das et al. 2014; Kim et al. 2010; Loh et al. 2007; Wang et al. 2010). Overexpression of KDM4 is linked to more aggressive disease and poorer clinical outcomes (Jia et al. 2020; Bur et al. 2016; Soini et al. 2015). Functional redundancy and cross-activity have been observed across KDM4 isoforms; selective inhibition of one isoform appears to not be effective (Agger et al. 2016; Pedersen et al. 2016).

Heterocyclic KDM4 Inhibitor

[0067] The heterocyclic KDM4 inhibitor described herein refers to Compound 1 having the structure below, and the chemical name 3-({[(4R)-7-{methyl[4-(propan-2-yl)phenyl]amino}-3,4-dihydro-2H-1-benzopyran-4-yl]methyl}amino)pyridine-4-carboxylic acid:



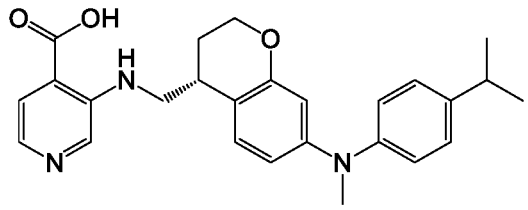
Compound 1.

[0068] Compound 1 has been previously disclosed in PCT patent publication WO2015/200709 and related patent applications and granted patents, such as US 9,242,968, which are incorporated by reference in their entirety. Compound 1 is a pan inhibitor of KDM4 that simultaneously targets multiple isoforms of KDM4. Throughout this disclosure when reference is made to a heterocyclic KDM4 inhibitor, or pharmaceutically acceptable salts or solvates thereof, the reference is to Compound 1.

Cancer and Methods of Treatment

[0069] In one embodiment described herein is a method for inhibiting a histone-demethylase enzyme comprising contacting the enzyme with Compound 1 as disclosed herein, wherein the histone-demethylase enzyme comprises KDM4. In certain aspects, disclosed herein is a method of treating a cancer in an individual in need thereof, comprising administering an effective amount of a heterocyclic KDM4 inhibitor described herein to the individual. In certain aspects, disclosed herein is a heterocyclic KDM4 inhibitor described herein for use in treating a cancer. In certain aspects, disclosed herein is a heterocyclic KDM4 inhibitor described herein for use in preparation of a medicament for treating a cancer.

[0070] One embodiment provides a method of treating a cancer in a cancer patient in need thereof, comprising administering to the individual a compound, or pharmaceutically acceptable salt or solvate thereof, having the structure:



. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from colorectal cancer, esophageal cancer, triple negative breast cancer, gastric cancer, lymphoma, gastric adenocarcinoma, diffuse large B-cell non-Hodgkin's lymphoma, acute T-cell leukemia, esophageal squamous cell carcinoma, multiple myeloma, acute myeloid leukemia, colorectal adenocarcinoma, colorectal carcinoma, pancreatic cancer, pancreatic carcinoma, breast carcinoma, or T-cell acute lymphoblastic leukemia.

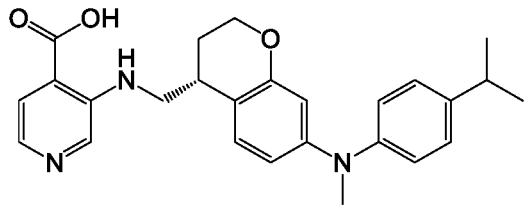
[0071] Another embodiment provides the method, wherein the cancer patient has been diagnosed with colorectal cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with esophageal cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with triple negative breast cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with gastric cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with lymphoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with gastric adenocarcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with diffuse large B-cell non-Hodgkin's lymphoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with acute T-cell leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from esophageal squamous cell carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with multiple myeloma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with acute myeloid leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with colorectal adenocarcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with colorectal carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with pancreatic cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with pancreatic carcinoma. Another embodiment provides the

method, wherein the cancer patient has been diagnosed with breast carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with T-cell acute lymphoblastic leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from lung cancer, small cell lung cancer, non-small cell lung cancer, large cell lung cancer, lung adenocarcinoma, lung squamous cell carcinoma, lung small cell carcinoma, lung large cell carcinoma, or bronchioloalveolar adenocarcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from acute lymphoblastic B-cell leukemia, mantle cell lymphoma, plasma cell myeloma, diffuse large B-cell lymphoma, B-cell lymphoma, Burkitt lymphoma, blast phase chronic myeloid leukemia.

[0072] Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from intestinal cancer, intestinal adenocarcinoma, squamous cell carcinoma of the upper digestive tract. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from stomach cancer, stomach signet ring adenocarcinoma, adenocarcinoma of the stomach, or adenosquamous carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from ovarian cancer, ovarian endometrioid carcinoma, ovarian clear cell carcinoma, ovarian adenocarcinoma, endometrial cancer, endometrial adenocarcinoma, prostate cancer, or prostate adenocarcinoma.

[0073] Another embodiment provides the method, wherein the cancer patient has been diagnosed with skin cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from thyroid cancer, or thyroid follicular carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from breast cancer, or breast ductal carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from liver cancer, or hepatocellular carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from a CNS cancer, astrocytoma grade IV, or gliosarcoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with bone cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from kidney cancer, clear cell renal cell carcinoma, renal cell carcinoma, urinary tract cancer, or urinary tract transitional cell carcinoma. Another embodiment provides the method, wherein the cancer is relapsed after prior therapy, refractory to prior therapy, or acquired resistance to prior therapy.

[0074] Another embodiment provides a method of reducing tumorigenic cell population in cancer patient in need thereof, comprising administering to the individual a compound, or pharmaceutically acceptable salt or solvate thereof, having the structure:



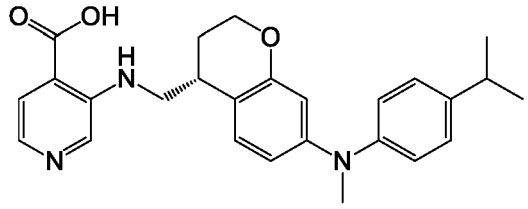
. Another embodiment provides the method, wherein the population of tumorigenic cell is reduced from about 1.5-fold to about 100-fold. Another embodiment provides the method, wherein the population of tumorigenic cell is reduced about 1.5-fold, about 2.0-fold, about 2.5-fold, about 3.0-fold, about 3.5-fold, about 4.0-fold, about 4.5-fold, about 5.0-fold, about 6.0-fold, about 7.0-fold, about 8.0-fold, about 9.0-fold, about 10-fold, about 15-fold, about 20-fold, about 25-fold, about 30-fold, about 35-fold, about 40-fold, about 45-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, or about 100-fold. Another embodiment provides the method, wherein the population of tumorigenic cell is reduced about 1.5-fold, about 2.0-fold, about 2.5-fold, about 3.0-fold, about 3.5-fold, about 4.0-fold, about 4.5-fold, or about 5.0-fold. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from colorectal cancer, esophageal cancer, triple negative breast cancer, gastric cancer, lymphoma, gastric adenocarcinoma, diffuse large B-cell non-Hodgkin's lymphoma, acute T-cell leukemia, esophageal squamous cell carcinoma, multiple myeloma, acute myeloid leukemia, colorectal adenocarcinoma, colorectal carcinoma, pancreatic cancer, pancreatic carcinoma, breast carcinoma, or T-cell acute lymphoblastic leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with colorectal cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with esophageal cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with triple negative breast cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with gastric cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with lymphoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with gastric adenocarcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with diffuse large B-cell non-Hodgkin's lymphoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with acute T-cell leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with esophageal squamous cell carcinoma. Another

embodiment provides the method, wherein the cancer patient has been diagnosed with multiple myeloma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with acute myeloid leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with colorectal adenocarcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with colorectal carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with pancreatic cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with pancreatic carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with breast carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with T-cell acute lymphoblastic leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from lung cancer, small cell lung cancer, non-small cell lung cancer, large cell lung cancer, lung adenocarcinoma, lung squamous cell carcinoma, lung small cell carcinoma, lung large cell carcinoma, or bronchioloalveolar adenocarcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from acute lymphoblastic B-cell leukemia, mantle cell lymphoma, plasma cell myeloma, diffuse large B-cell lymphoma, B-cell lymphoma, Burkitt lymphoma, blast phase chronic myeloid leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from intestinal cancer, intestinal adenocarcinoma, squamous cell carcinoma of the upper digestive tract. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from stomach cancer, stomach signet ring adenocarcinoma, adenocarcinoma of the stomach, or adenosquamous carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from ovarian cancer, ovarian endometrioid carcinoma, ovarian clear cell carcinoma, ovarian adenocarcinoma, endometrial cancer, endometrial adenocarcinoma, prostate cancer, or prostate adenocarcinoma.

[0075] Another embodiment provides the method, wherein the cancer patient has been diagnosed with skin cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from thyroid cancer, or thyroid follicular carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from breast cancer, or breast ductal carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from liver cancer, or hepatocellular carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from a CNS

cancer, astrocytoma grade IV, or gliosarcoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with bone cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from kidney cancer, clear cell renal cell carcinoma, renal cell carcinoma, urinary tract cancer, or urinary tract transitional cell carcinoma. Another embodiment provides the method, wherein the cancer is relapsed after prior therapy, refractory to prior therapy, or acquired resistance to prior therapy.

[0076] Another embodiment provides a method of reducing tumor initiating cell frequency in a cancer patient in need thereof, comprising administering to the individual a compound, or pharmaceutically acceptable salt or solvate thereof, having the structure:



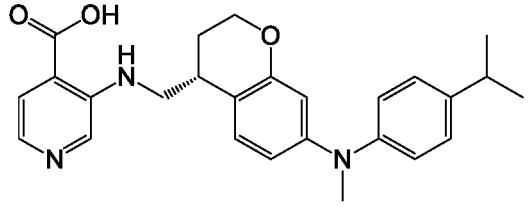
. Another embodiment provides the method, wherein the tumor initiating cell frequency is reduced from about 1.5-fold to about 100-fold. Another embodiment provides the method, wherein the tumor initiating cell frequency is reduced about 1.5-fold, about 2.0-fold, about 2.5-fold, about 3.0-fold, about 3.5-fold, about 4.0-fold, about 4.5-fold, about 5.0-fold, about 6.0-fold, about 7.0-fold, about 8.0-fold, about 9.0-fold, or about 10-fold. Another embodiment provides the method, wherein the tumor initiating cell frequency is reduced to a frequency of about 1-in-100 cells to about 1-in-10,000 cells. Another embodiment provides the method, wherein the tumor initiating cell frequency is reduced to a frequency of about 1-in-100 cells, about 1-in-200 cells, about 1-in-300 cells, about 1-in-400 cells, about 1-in-500 cells, about 1-in-600 cells, about 1-in-700 cells, about 1-in-800 cells, about 1-in-900 cells, about 1-in-1,000 cells, about 1-in-1,200 cells, about 1-in-1,400 cells, about 1-in-1,600 cells, about 1-in-1,800 cells, about 1-in-2,000 cells, about 1-in-3,000 cells, about 1-in-4,000 cells, about 1-in-5,000 cells, about 1-in-6,000 cells, about 1-in-7,000 cells, about 1-in-8,000 cells, about 1-in-9,000 cells, or to about 1-in-10,000 cells. Another embodiment provides the method, wherein the tumor initiating cell frequency is reduced to a frequency of about 1-in-200 cells, about 1-in-300 cells, about 1-in-400 cells, about 1-in-500 cells, about 1-in-600 cells, about 1-in-700 cells, about 1-in-800 cells, about 1-in-900 cells, about 1-in-1,000 cells, or about 1-in-1,200 cells. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from colorectal cancer, esophageal cancer, triple negative breast cancer, gastric cancer, lymphoma, gastric adenocarcinoma, diffuse large B-cell non-Hodgkin's lymphoma, acute T-cell leukemia, esophageal squamous cell carcinoma, multiple myeloma,

acute myeloid leukemia, colorectal adenocarcinoma, colorectal carcinoma, pancreatic cancer, pancreatic carcinoma, breast carcinoma, or T-cell acute lymphoblastic leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with colorectal cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with esophageal cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with triple negative breast cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with gastric cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with lymphoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from gastric adenocarcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with diffuse large B-cell non-Hodgkin's lymphoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with acute T-cell leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with esophageal squamous cell carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with multiple myeloma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with acute myeloid leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with colorectal adenocarcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with colorectal carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with pancreatic cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with pancreatic carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with breast carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with T-cell acute lymphoblastic leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from lung cancer, small cell lung cancer, non-small cell lung cancer, large cell lung cancer, lung adenocarcinoma, lung squamous cell carcinoma, lung small cell carcinoma, lung large cell carcinoma, or bronchioloalveolar adenocarcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from acute lymphoblastic B-cell leukemia, mantle cell lymphoma, plasma cell myeloma, diffuse large B-cell lymphoma, B-cell lymphoma, Burkitt lymphoma, blast phase chronic myeloid leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from intestinal cancer, intestinal adenocarcinoma, squamous cell carcinoma of the upper digestive tract. Another embodiment

provides the method, wherein the cancer patient has been diagnosed with a cancer selected from stomach cancer, stomach signet ring adenocarcinoma, adenocarcinoma of the stomach, or adenosquamous carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from ovarian cancer, ovarian endometrioid carcinoma, ovarian clear cell carcinoma, ovarian adenocarcinoma, endometrial cancer, endometrial adenocarcinoma, prostate cancer, or prostate adenocarcinoma.

[0077] Another embodiment provides the method, wherein the cancer patient has been diagnosed with skin cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from thyroid cancer, or thyroid follicular carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from breast cancer, or breast ductal carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from liver cancer, or hepatocellular carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from a CNS cancer, astrocytoma grade IV, or gliosarcoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with bone cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from kidney cancer, clear cell renal cell carcinoma, renal cell carcinoma, urinary tract cancer, or urinary tract transitional cell carcinoma. Another embodiment provides the method, wherein the cancer is relapsed after prior therapy, refractory to prior therapy, or acquired resistance to prior therapy.

[0078] Another embodiment provides a method of inhibiting cancer stem cells in a cancer patient in need thereof, comprising administering to the individual a compound, or pharmaceutically acceptable salt or solvate thereof, having the structure:



. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from colorectal cancer, esophageal cancer, triple negative breast cancer, gastric cancer, lymphoma, gastric adenocarcinoma, diffuse large B-cell non-Hodgkin's lymphoma, acute T-cell leukemia, esophageal squamous cell carcinoma, multiple myeloma, acute myeloid leukemia, colorectal adenocarcinoma, colorectal carcinoma, pancreatic cancer, pancreatic carcinoma, breast carcinoma, or T-cell acute lymphoblastic leukemia. Another embodiment provides the method, wherein the cancer patient

has been diagnosed with colorectal cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with esophageal cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with triple negative breast cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with gastric cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with lymphoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with gastric adenocarcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with diffuse large B-cell non-Hodgkin's lymphoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with acute T-cell leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with esophageal squamous cell carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with multiple myeloma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with acute myeloid leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with colorectal adenocarcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with colorectal carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with pancreatic cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with pancreatic carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with breast carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with T-cell acute lymphoblastic leukemia. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from lung cancer, small cell lung cancer, non-small cell lung cancer, large cell lung cancer, lung adenocarcinoma, lung squamous cell carcinoma, lung small cell carcinoma, lung large cell carcinoma, or bronchioloalveolar adenocarcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from acute lymphoblastic B-cell leukemia, mantle cell lymphoma, plasma cell myeloma, diffuse large B-cell lymphoma, B-cell lymphoma, Burkitt lymphoma, blast phase chronic myeloid leukemia.

Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from intestinal cancer, intestinal adenocarcinoma, squamous cell carcinoma of the upper digestive tract. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from stomach cancer, stomach signet ring adenocarcinoma, adenocarcinoma of the stomach, or adenosquamous carcinoma. Another

embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from ovarian cancer, ovarian endometrioid carcinoma, ovarian clear cell carcinoma, ovarian adenocarcinoma, endometrial cancer, endometrial adenocarcinoma, prostate cancer, or prostate adenocarcinoma.

[0079] Another embodiment provides the method, wherein the cancer patient has been diagnosed with skin cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from thyroid cancer, or thyroid follicular carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from breast cancer, or breast ductal carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from liver cancer, or hepatocellular carcinoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from a CNS cancer, astrocytoma grade IV, or gliosarcoma. Another embodiment provides the method, wherein the cancer patient has been diagnosed with bone cancer. Another embodiment provides the method, wherein the cancer patient has been diagnosed with a cancer selected from kidney cancer, clear cell renal cell carcinoma, renal cell carcinoma, urinary tract cancer, or urinary tract transitional cell carcinoma. Another embodiment provides the method, wherein the cancer is relapsed after prior therapy, refractory to prior therapy, or acquired resistance to prior therapy.

Microsatellite Instability (MSI-H) Status

[0080] Microsatellite (MS), also called Short Tandem Repeats (STRs) or Simple Sequence Repeat (SSRs), consists of repeated sequences of 1–6 nucleotides (*see*, *e.g.*, Garrido-Ramos (2017) Genes 8(9):230). The distribution characteristics of MS are different from 15 to 65 nucleotides tandem repeats of small satellite DNA. Depending on the frequency of MSI, it can be distinguished into three types: high microsatellite instability (MSI-H), low microsatellite instability (MSI-L) and microsatellite stability (MSS) (*see*, *e.g.*, Li, et al., (2020) Cancer Cell International 20:16). Due to deficiency of mismatch repair (MMR) genes in tumor cells or defects in the process of replication repair, the patients with high microsatellite instability (MSI-H) tumors may show higher sensitivity to treatments and benefit from immunotherapy.

[0081] In certain embodiments of the methods provided herein, the method further comprises determining high microsatellite instability (MSI-H) status of the cancer patient. In certain embodiments of the methods provided herein, the method further comprises determining whether the cancer patient is diagnosed with high microsatellite instability (MSI-H).

[0082] In certain embodiments of the methods provided herein, the cancer patient has been determined to have a high microsatellite instability (MSI-H) status. In certain embodiments of

the methods provided herein, the cancer patient has been diagnosed with a cancer of high microsatellite instability (MSI-H).

[0083] Another embodiment provides a method of treating a cancer in a cancer patient in need thereof, comprising (a) determining high microsatellite instability (MSI-H) status of the cancer patient, and (b) administering therapeutically effective amount of Compound 1, or a pharmaceutically acceptable salt or a solvate thereof to the cancer patient if the cancer patient is determined to be a MSI-H cancer patient. In some embodiments, the cancer patient has been diagnosed with colorectal cancer. In some embodiments, the cancer patient has been diagnosed with colorectal cancer with MSI-H status.

[0084] Another embodiment provides a method of treating a cancer having a high microsatellite instability (MSI-H) status in a cancer patient in need thereof, comprising administering therapeutically effective amount of Compound 1, or a pharmaceutically acceptable salt or a solvate thereof to a patient that is determined to be a MSI-H cancer patient. In some embodiments, the cancer patient has been diagnosed with colorectal cancer.

[0085] Another embodiment provides a method of treating a high microsatellite instability (MSI-H) cancer patient, comprising administering therapeutically effective amount of Compound 1, or a pharmaceutically acceptable salt or a solvate thereof to a patient that is determined to be a MSI-H cancer patient. In some embodiments, the cancer patient has been diagnosed with colorectal cancer.

[0086] Another embodiment provides a method of reducing tumorigenic cell population in cancer patient in need thereof, comprising (a) determining high microsatellite instability (MSI-H) status of the cancer patient, and (b) administering therapeutically effective amount of Compound 1, or a pharmaceutically acceptable salt or a solvate thereof to a patient that is determined to be a MSI-H cancer patient. In some embodiments, the cancer patient has been diagnosed with colorectal cancer. In some embodiments, the cancer patient has been diagnosed with colorectal cancer with MSI-H status.

[0087] Another embodiment provides a method of reducing tumor initiating cell frequency in a cancer patient in need thereof, comprising (a) determining high microsatellite instability (MSI-H) status of the cancer patient, and (b) administering therapeutically effective amount of Compound 1, or a pharmaceutically acceptable salt or a solvate thereof to a patient that is determined to be a MSI-H cancer patient. In some embodiments, the cancer patient has been diagnosed with colorectal cancer. In some embodiments, the cancer patient has been diagnosed with colorectal cancer with MSI-H status.

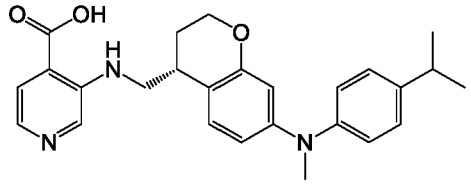
[0088] Another embodiment provides a method of inhibiting cancer stem cells in a cancer patient in need thereof, comprising (a) determining high microsatellite instability (MSI-H) status of the cancer patient, and (b) administering therapeutically effective amount of Compound 1, or a pharmaceutically acceptable salt or a solvate thereof to a patient that is determined to be a MSI-H cancer patient. In some embodiments, the cancer patient has been diagnosed with colorectal cancer. In some embodiments, the cancer patient has been diagnosed with colorectal cancer with MSI-H status.

[0089] Microsatellite instability (MSI-H) status can be detected or determined using methods known to those skilled in the art, including but not limited to Next-Generation Sequencing (NGS), fluorescent multiplex Polymerase Chain Reaction (PCR), capillary electrophoresis (CE), immunohistochemistry (IHC), single-molecule molecular inversion probes (smMIPs), and MSI calculation (e.g., MANTIS). In one embodiment, MSI-H status is determined by Next-Generation Sequencing (NGS). In another embodiment, MSI-H status is determined by fluorescent multiplex PCR. In yet another embodiment, MSI-H status is determined by capillary electrophoresis (CE). In yet another embodiment, MSI-H status is determined by immunohistochemistry (IHC). In yet another embodiment, MSI-H status is determined by single-molecule molecular inversion probes (smMIPs). In yet another embodiment, MSI-H status is determined by MSI calculation.

[0090] In certain embodiments, the MSI-H has two or more repeat loci microsatellite markers selected from the group consisting of BAT-25, BAT-26, D2S123, D5S346 and D17S250. In certain embodiments, the cancer patient is determined to have a MSI-H status when the cancer patient is determined to have two or more repeat loci microsatellite markers selected from the group consisting of BAT-25, BAT-26, D2S123, D5S346 and D17S250.

Biomarkers and Methods of Use Thereof

[0091] The methods provided herein are based, in part, on the finding that detectable increase or decrease in certain biomarkers upon compound treatment are observed in cell culture and organoids of cancer (e.g., colorectal cancer), responsive to a given treatment, *e.g.*, Compound 1 having a structure of:



,

or a pharmaceutically acceptable salt or solvate thereof, and that the levels of these biomarkers may be used for predicting the responsiveness of the patients to the treatment.

[0092] A "biological marker" or "biomarker" is a substance, the change and/or the detection of which indicates a particular biological state. In some embodiments, the indication is the responsiveness of a disease, e.g., cancer (e.g., colorectal cancer), to a given treatment (e.g., Compound 1 or a pharmaceutically acceptable salt or solvate thereof).

[0093] As described in the Examples and shown in the figures, the levels of certain proteins and/or mRNAs change in response to Compound 1. These biomarkers include PNUTS (PPP1R10), ANK1, IBA57, SOWAHD, MF12-AS1, and CECR1. Each of the biomarkers provided herein includes various isoforms, phosphorylated forms, cleaved forms, modified forms, and splicing variants thereof. In some embodiments, the levels of the isoforms, phosphorylated forms, cleaved forms, modified forms, and/or splicing variants of these biomarkers increase or decrease in response to the compound treatment, and thus these isoforms, phosphorylated forms, cleaved forms, modified forms, and/or splicing variants of the biomarkers can be used to predict a patient's response.

[0094] In one aspect, provided herein are methods of identifying a cancer patient with a cancer likely to be responsive to a treatment compound, comprising: (a) administering the treatment compound to the patient; (b) obtaining a sample from the patient; (c) determining the level of a biomarker in the sample; and (d) diagnosing the patient as being likely to be responsive to the treatment compound if the level of the biomarker in the sample is different from a reference level of the biomarker; wherein the treatment compound is Compound 1, or a pharmaceutically acceptable salt or a solvate thereof. In one embodiment, the biomarker is PNUTS.

[0095] In another aspect, provided herein are methods of identifying a cancer patient having cancer who is likely to be responsive to a treatment compound, comprising: (a) obtaining a sample from the patient; (b) administering the treatment compound to the sample; (c) determining the level of a biomarker in the sample; and (d) diagnosing the patient as being likely to be responsive to the treatment compound if the level of the biomarker in the sample is different from a reference level of the biomarker; wherein the treatment compound is Compound 1, or a pharmaceutically acceptable salt or a solvate thereof. In one embodiment, the biomarker is PNUTS.

[0096] In some embodiments of the methods provided herein, administering a treatment compound to the sample from the cancer patient is performed *in vitro*. In other embodiments, administering a treatment compound to the sample from the patient having cancer is performed *in vivo*. In certain embodiments, the sample is cells. In one embodiment, the cells are contacted with the treatment compound for a period of time, e.g., 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, or 55 minutes, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24

hours, or 2, 3, or more days. In other embodiments, the cells are obtained from another cancer patient.

[0097] In some embodiments, the level of the biomarker in the sample of the patient is higher than the reference level of the biomarker. In other embodiments, the level of the biomarker in the sample of the patient is lower than the reference level of the biomarker.

[0098] In yet another aspect, provided herein are methods of predicting the responsiveness of a cancer patient to a treatment compound, comprising: (a) administering the treatment compound to the patient; (b) obtaining a sample from the patient; (c) determining the level of PNUTS, ANK1, IBA57, SOWAHD, MF12-AS1, or CECR1 in the sample; (d) diagnosing the patient as being likely to be responsive to a treatment of the cancer with the treatment compound if the level of PNUTS, ANK1, IBA57, SOWAHD, MF12-AS1, or CECR1 in the sample is different than the level of PNUTS, ANK1, IBA57, SOWAHD, MF12-AS1,or CECR1 obtained from a reference sample; wherein the treatment compound is Compound 1, or a pharmaceutically acceptable salt or a solvate thereof. In one embodiment, the biomarker is PNUTS.

[0099] In yet another aspect, provided herein is a method of predicting the responsiveness of a cancer patient to a treatment compound, comprising: (a) obtaining a sample from the patient; (b) administering the treatment compound to the sample; (c) determining the level of PNUTS, ANK1, IBA57, SOWAHD, MF12-AS1, or CECR1 in the sample; and (d) diagnosing the patient as being likely to be responsive to a treatment of the cancer with the treatment compound if the level of PNUTS, ANK1, IBA57, SOWAHD, MF12-AS1, or CECR1 in the sample is different than the level of PNUTS, ANK1, IBA57, SOWAHD, MF12-AS1, or CECR1 obtained from a reference sample; wherein the treatment compound is Compound 1, or a pharmaceutically acceptable salt or a solvate thereof. In one embodiment, the level of PNUTS is determined and compared.

[00100] In yet another aspect, provided herein are methods of monitoring the efficacy of a treatment of cancer in a subject with a treatment compound, comprising: (a) administering the treatment compound to the patient; (b) obtaining a sample from the patient; (c) determining the level of PNUTS, ANK1, IBA57, SOWAHD, MF12-AS1, or CECR1 in the sample; (d) diagnosing the patient as being likely to be responsive to a treatment of the cancer with the treatment compound if the level of PNUTS, ANK1, IBA57, SOWAHD, MF12-AS1, or CECR1 in the sample is different than the level of PNUTS, ANK1, IBA57, SOWAHD, MF12-AS1,or CECR1 obtained from a reference sample; wherein the treatment compound is Compound 1, or a pharmaceutically acceptable salt or a solvate thereof. In some embodiments, a change in the level of the biomarker as compared to the reference is indicative of the efficacy of the treatment

compound in treating the cancer in the patient. In one embodiment, an increased level of the biomarker is indicative of the efficacy of the treatment compound in treating the cancer in the patient. In another embodiment, a decreased level of the biomarker is indicative of the efficacy of the treatment compound in treating the cancer in the patient. In one embodiment, the level of PNUTS is determined and compared.

[00101] In certain embodiments, when the cancer patient is diagnosed as being likely to be responsive to the treatment compound, the methods provided herein further comprise administering a therapeutically effective amount of the treatment compound to the patient.

[00102] In another aspect, provided herein are methods of treating cancer, comprising: (a) obtaining a sample from a patient having the cancer; (b) determining the level of a biomarker in the sample; (c) diagnosing the patient as being likely to be responsive to a treatment compound if the level of the biomarker in the sample is different from a reference level of the biomarker; and (d) administering a therapeutically effective amount of the treatment compound to the patient; wherein the treatment compound is Compound 1, or a pharmaceutically acceptable salt or a solvate thereof. In one embodiment, the biomarker is PNUTS.

[00103] In some embodiments, the level of the biomarker in the sample of the patient is higher than the reference level of the biomarker. In other embodiments, the level of the biomarker in the sample of the patient is lower than the reference level of the biomarker.

[00104] In some embodiments of the various methods provided herein, a treatment compound is administered to a patient likely to be responsive to the treatment compound. Also provided herein are methods of treating patients who have been previously treated for cancer but are non-responsive to standard therapies, as well as those who have not previously been treated. The invention also encompasses methods of treating patients regardless of patient's age, although some diseases or disorders are more common in certain age groups. The invention further encompasses methods of treating patients who have undergone surgery in an attempt to treat the disease or condition at issue, as well as those who have not. Because patients with cancer have heterogeneous clinical manifestations and varying clinical outcomes, the treatment given to a patient may vary, depending on his/her prognosis. The skilled clinician will be able to readily determine without undue experimentation specific secondary agents, types of surgery, and types of non-drug based standard therapy that can be effectively used to treat an individual patient with cancer.

[00105] As shown in the Examples, the levels of certain biomarkers change in response to Compound 1 treatment, such as PNUTS (PPP1R10), ANK1, IBA57, SOWAHD, MF12-AS1, and CECR1. Thus, in some embodiments, the biomarker is selected from the group consisting of

PNUTS (PPP1R10), ANK1, IBA57, SOWAHD, MF12-AS1, and CECR1. In one embodiment, the biomarker is PNUTS (PPP1R10). In another embodiment, the biomarker is ANK1. In yet another embodiment, the biomarker is IBA57. In yet another embodiment, the biomarker is SOWAHD. In yet another embodiment, the biomarker is MF12-AS1. In yet another embodiment, the biomarker is CECR1.

[00106] In some embodiments of the various methods provided herein, the biomarker is PNUTS, ANK1, or IBA57, and wherein the level of PNUTS, ANK1, or IBA57 decreases as compared to a reference in response to Compound 1 treatment. In some embodiments of the various methods provided herein, the biomarker is SOWAHD, MF12-AS1, or CECR1 and the level of SOWAHD, MF12-AS1, or CECR1 increases as compared to a reference in response to Compound 1 treatment.

[00107] In certain embodiments, the various methods provided herein use samples (e.g., biological samples) from subjects or individuals (e.g., patients). The patient can be a patient, such as, a patient with a cancer (e.g., colorectal cancer). The patient can be male or female, and can be an adult, a child, or an infant. Samples can be analyzed at a time during an active phase of a cancer (e.g., colorectal cancer), or when the cancer (e.g., colorectal cancer) is inactive. In certain embodiments, more than one sample from a patient can be obtained.

[00108] In some embodiments, the sample used in the present methods comprises a biopsy (e.g., a tumor biopsy). The biopsy can be from any organ or tissue, for example, skin, liver, lung, heart, colon, kidney, bone marrow, teeth, lymph node, hair, spleen, brain, breast, or other organs. Any biopsy technique known by those skilled in the art can be used for isolating a sample from a subject, for instance, open biopsy, close biopsy, core biopsy, incisional biopsy, excisional biopsy, or fine needle aspiration biopsy.

[00109] In one embodiment, the sample used in the methods provided herein is obtained from the patient prior to the patient receiving a treatment for the disease or disorder. In another embodiment, the sample is obtained from the patient during the patient receiving a treatment for the disease or disorder. In another embodiment, the sample is obtained from the patient after the patient has received a treatment for the disease or disorder. In various embodiments, the treatment comprises administering Compound 1 to the patient.

[00110] In certain embodiments, the sample used in the methods provided herein comprises a plurality of cells, such as cancer (e.g., colorectal cancer) cells. Such cells can include any type of cells, e.g., stem cells, blood cells (e.g., peripheral blood mononuclear cells (PBMC)), lymphocytes, B cells, T cells, monocytes, granulocytes, immune cells, or cancer cells.

Pharmaceutical Compositions

[00111] In certain embodiments, the heterocyclic KDM4 inhibitor described herein is administered as a pure chemical. In other embodiments, the heterocyclic KDM4 inhibitor described herein is combined with a pharmaceutically suitable or acceptable carrier (also referred to herein as a pharmaceutically suitable or acceptable excipient, a physiologically suitable or acceptable excipient, or a physiologically suitable or acceptable carrier) selected on the basis of a chosen route of administration and standard pharmaceutical practice.

[00112] Provided herein is a pharmaceutical composition comprising the heterocyclic KDM4 inhibitor as described herein, or a stereoisomer, pharmaceutically acceptable salt, hydrate, or solvate thereof, together with one or more pharmaceutically acceptable carriers. The carrier(s) (or excipient(s)) is acceptable or suitable if the carrier is compatible with the other ingredients of the composition and not deleterious to the recipient (*i.e.,* the subject or the patient) of the composition.

[00113] One embodiment provides a method of preparing a pharmaceutical composition comprising mixing the heterocyclic KDM4 inhibitor as described herein, or a stereoisomer, pharmaceutically acceptable salt, hydrate, or solvate thereof, and a pharmaceutically acceptable carrier.

[00114] Provided herein is the method wherein the pharmaceutical composition is administered orally. Suitable oral dosage forms include, for example, tablets, pills, sachets, or capsules of hard or soft gelatin, methylcellulose or of another suitable material easily dissolved in the digestive tract. In some embodiments, suitable nontoxic solid carriers are used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. (*See*, *e.g.*, *Remington: The Science and Practice of Pharmacy* (Gennaro, 21st Ed. Mack Pub. Co., Easton, PA (2005)).

[00115] Provided herein is the method wherein the pharmaceutical composition is administered by injection. In some embodiments, the heterocyclic KDM4 inhibitor as described herein, or pharmaceutically acceptable salt or solvate thereof, is formulated for administration by injection. In some instances, the injection formulation is an aqueous formulation. In some instances, the injection formulation is a non-aqueous formulation. In some instances, the injection formulation is an oil-based formulation, such as sesame oil, or the like.

[00116] The dose of the composition comprising the heterocyclic KDM4 inhibitor as described herein, or a stereoisomer, pharmaceutically acceptable salt, hydrate, or solvate thereof, differs depending upon the subject or patient's (e.g., human) condition. In some embodiments,

such factors include general health status, age, and other factors. Pharmaceutical compositions are administered in a manner appropriate to the disease to be treated (or prevented). An appropriate dose and a suitable duration and frequency of administration will be determined by such factors as the condition of the patient, the type and severity of the patient's disease, the particular form of the active ingredient, and the method of administration. In general, an appropriate dose and treatment regimen provides the composition(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit (e.g., an improved clinical outcome, such as more frequent complete or partial remissions, or longer disease-free and/or overall survival, or a lessening of symptom severity. Optimal doses are generally determined using experimental models and/or clinical trials. The optimal dose depends upon the body mass, weight, or blood volume of the patient.

EXAMPLES

[00117] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

BACKGROUND

[00118] The KDM4 family of histone lysine demethylases consists of four main isoforms (KDM4A,B, C, D), all of which have been identified as key oncogenic drivers. They function as epigenetic regulators and control transitions between transcriptionally silent and active chromatin states via removal of methyl marks on histone H3K9 and histone H3K36. KDM4 isoforms play an important role in the epigenetic dysregulation in various cancers and islinked to more aggressive disease and poorer clinical outcomes. Functional redundancy and cross-activity have been observed across KDM4 family members, thus, selective inhibition of one isoform appears to not be effective. Compound 1 is a pan inhibitor of KDM4 that simultaneously targets multiple isoforms of KDM4.

Example 1: KDM4 inhibitory activity of Compound 1

[00119] Fig. 1 illustrates the biochemical activities of Compound 1, a potent PAN KDM4 inhibitor. Fig. 2 illustrates the reversible and competitive inhibition of H3K9me3 demethylation. Fig. 3 illustrates that compound 1 induces cell cycle arrest in S-phase. Fig. 4 demonstrates that Compound 1 induces apoptosis in human cancer cell lines. *See also*: PCT patent publication WO2015/200709 and related patent applications and granted patents, such as US 9,242,968, which are incorporated by reference in their entirety.

Example 2a: Compound 1 inhibits cell proliferation in cell culture and organoid models

[00120] Compound 1 was evaluated in *in vitro* cell-proliferation assays (BrdU assay, MTS assay, orCell Titer Glo (CTG) assay) in multiple cancer cell lines and patient-derived organoid models to determine half-maximal inhibitory concentrations (IC50s).

[00121] Viability and BrdU thymidine incorporation assays were performed in multiwell plates seeded with cells in growth medium. After seeding, multiwell plates were cultured for 24 hours in a humidified incubator at 37 °C to promote adherence. The assay was initiated in individual test wells by adding either DMSO as a negative control, or an inhibitor panobinostat at ≥ 100x IC50 concentration as a positive control, or serially diluted Compound 1. The cultures were incubated for 168 hours after which the number of viable cells or the amount of thymidine incorporation in each test well was assessed by the methods as indicated above. Readouts were performed using an EnVision® Multilabel Reader (PerkinElmer, Waltham, MA) and the results were expressed as a percent of the negative control. Percent of Control values were plotted against the corresponding Compound 1 concentration and the relative IC50 value was determined from a non-linear regression curve as the concentration where inhibition was half-maximal. For cell lines where the number of IC50 determinations exceeded one, the IC50 was expressed as the mean IC50 ± SD.

[00122] Half-maximal inhibitory concentration (IC50) values for Compound 1 inhibition of cell proliferation were determined for nine cancer cell lines and one normal human fibroblastcell line. As shown in Table 1, except for IMR-90, a normal human fibroblast line, and ZR-75-1, a human breast carcinoma line, Compound 1 treatment for 168 hours demonstrated potent anti-proliferative activity across the panel, which included both solid and hematological cancer cell lines. Compound 1 showed potent anti-proliferative activity (< 0.040 μM) for eight solid and hematological cancer cell lines: Jurkat, MDA-MB-231, KYSE-150, MM.1s, HL-60, HT-29, MCF-7, and Loucy.

Table 1

Cell Line	Description	IC_{50} (μM)	SD (μM)	N(n)	Assay Format		
					BrdU	MTS	CTG
Jurkat	human acute T cell leukemia	0.0027	0.0022	4(2)	1	2	1
MDA-MB-231	human triple negative breast cancer	0.0035	0.0050	4(3)	4	---	---
KYSE-150	human esophageal squamous cell carcinoma	0.0053	0.0040	8(3)	5	3	---
MM.1s	human multiple myeloma	0.0090	---	1(1)	---	1	---
HL-60	human acute myeloid leukemia	0.0130	---	1(1)	---	1	---
HT-29	human colorectal adenocarcinoma	0.0235	0.0134	2(2)	1	---	1
MCF-7	human breast carcinoma	0.0370	---	1(1)	---	1	---
Loucy	human T-acute lymphoblastic leukemia	<0.01	---	1(1)	---	1	---
IMR-90	(normal) human fetal lung fibroblast	>1.0	---	2(1)	1	---	1
ZR-75-1	human breast carcinoma	>10	---	1(1)	---	1	---

MTS = CellTiter 96® AQueous One Solution Cell Proliferation Assay, CTG = CellTiter-Glo® Luminescent Cell Viability Assay, and BrdU = 5-bromo-2′-deoxyuridine thymidine incorporation assay where the number indicates how many of each assay was performed.

[00123] The IC_{50} values for Compound 1 inhibition of colony formation and viability were determined for the patient-derived colon cancer organoid models: SU60, T002C, SU62, SU34, SU103,T035C, and SU106, patient-derived pancreatic cancer organoid models PA0165F, PA0143F, T016P, and T028P, and a patient-derived breast cancer organoid model FS53.

[00124] Colony formation and viability assays were performed in multiwell plates seeded with organoids using an *in vitro* culture system composed of a Matrigel layer, media containing a defined set of growth factors, and co-culture with Wnt3A-secreting mouse embryonic fibroblasts. Assays were initiated by adding either DMSO as a negative control, or an inhibitor panobinostat at ≥ 100x IC50 concentration as a positive control,or serially diluted Compound 1. The cultures were incubated for 168 or 144 hours after which the number of viable organoids in each test well was assessed respectively using one of two detection methods: CTG or staining with calcein-AM followed by imaging and colony counting. Colony count or viability of cells

treated with compound 1 was expressed as a percent of the negative control. Aggregated Percent of Control values were plotted against the corresponding Compound 1 concentration and the absolute IC50 value was determined from a non-linear regression curve as the concentration where inhibition was 50% of the control.

[00125] As shown in Table 2, Compound 1 treatment demonstrated sub-micromolar anti-proliferative activity for the colorectal carcinoma models SU60, T002C, and SU62 yielding IC50 values<0.15 M. Compound 1 treatment also demonstrated potent anti-proliferative activity for the PA0165F pancreatic carcinoma model yielding IC50 values <0.03 M. The SU34, SU103, T035C, and SU106 colorectal carcinoma models and the pancreatic carcinoma models PA0143F, T016P, and T028P were unresponsive to Compound 1 in these colony formation and viability assays with IC50 values >10 M. An IC50 value of 13.6 M was determined for the FS53 human breast carcinoma model following compound 1 treatment. A representative titration curve for data aggregated across two SU60 assays was constructed and presented as Fig. 9.

Table 2

Description	Organoid Model	IC_{50} (μM)	N	Assay Format	
				Time (hrs)	Readout
human colorectal carcinoma	SU60	0.023	2	168	CTG
		0.064	2	144	Colony Counts
	T002C	0.022	4	144	Colony Counts
	SU62	0.149	2	144	Colony Counts
	SU34	>10	4	144	Colony Counts
	SU103	>10	3	144	Colony Counts
	T035C	>10	3	144	Colony Counts
	SU106	>10	1	144	Colony Counts
human pancreatic carcinoma	PA0165F	0.029	1	168	CTG
		0.016	5	144	Colony Counts
	PA0143F	>10	4	144	Colony Counts
	T016P	>10	4	144	Colony Counts
	T028P	>10	6	144	Colony Counts
human breast carcinoma	FS53	13.6	2	144	Colony Counts

[00126] As shown in Fig. 9, percent of control = colony counts for Compound 1 treated organoids normalized to negative control colony counts, expressed as a percent; the curve combined data from two SU60 experiments; error bars are ±SD for two independent experiments. The colony formation inhibition assays were performed in multiwell plates seeded with SU60 organoids. The cultures were incubated for 168 hours after which the number of colonies was assessed by staining with calcein AM followed by imaging and colony counting. Colony counts were expressed as a percent of the negative control and aggregated Percent of

Control values were plotted against the corresponding Compound 1 concentration. A curve was fitted using non-linear regression.

Example 2b: Evaluation of Compound 1 in Cancer Cell Line Panel

[00127] In a larger screening study, compound 1 was evaluated across a broad cancer cell line panel (OncoPanel; HD Biosciences) composed of 301 cancer cell lines from different tumor types.

[00128] 2D cell viability inhibition assays were performed in multiwell plates seeded with cells from the panel. After seeding, the plates were cultured overnight in a humidified incubator at 37 °C to promote adherence. Assays were initiated in individual wells by adding either DMSO as a vehicle control, growth media as a blank, or serially diluted compound 1 (10 μM to 0.0005 μM with 1:3 serial dilution). Cultures were incubated for 168 hours after which the number of viable cells in each test well was assessed using the CellTiter-Glo® Luminescent Cell Viability Assay. Luminescence readouts were performed using an EnVision® Multilabel Reader and the compound 1 readouts were normalized to the DMSO control readouts, expressed as a percent of the control. Outliers were flagged out by visual inspection. Percent of control was plotted against the corresponding compound 1 concentration and the absolute IC50 value was determined using four-parameter logistic non-linear regression as the concentration where inhibition was 50% of the control. For maximum inhibition < 50%, the absolute IC50 was reported as > 10. In addition to EC50 and IC50, AUC was determined and was normalized to the area corresponding to theoretical no-inhibition (the rectangular area defined by the compound dose range and 0 to 100 in y). Emax was recorded as the minimum in y with the compound dose range. For those cell lines where Emax > 40, EC50 was manually set to 10 μM. A hierarchical clustering algorithm based on Euclidean distance between the standardized input values and "Ward.D" method as implemented in R hclust function was used to visualize the compound 1 cellular potency data. The sensitive/moderate/resistant calls for each cell line was determined by visually inspecting the resulting clustering dendrogram. All the analyses were done in R Core Team.

[00129] Results demonstrated that compound 1 potently inhibited proliferation of cancer cell lines and displayed a highly selective potency profile with absolute IC_{50} values ranging from < 500 pM to > 10 μM. Among the 301 cell lines, 209 were inhibited by compound 1 with IC_{50} values < 1 μM while 89 lines were insensitive with IC_{50} values >10 μM. Table 3 provides the results for all 301 cell lines.

Table 3

Cell line	*IC50(μM)*	*EC50(μM)*	*AUC*	*Emax*	*Tumor Type*	*Tumor Subtype*
NCIH1618	5.00E-04	0.000508	0.022662	0.488613	lung	small cell carcinoma
NCIH1048	0.000706	0.000508	0.147882	7.150228	lung	small cell carcinoma
NCIH1155	0.000765	0.000884	0.075713	0.26377	lung	large cell carcinoma
NCIH1666	0.001165	0.000508	0.298302	21.65869	lung	bronchioloalveolar adenocarcinoma
REH	0.00121	0.001324	0.095695	0.233625	haematopoietic and lymphoid tissue	acute lymphoblastic B cell leukaemia
MOLT4	0.00125	0.000508	0.137568	0.604781	haematopoietic and lymphoid tissue	acute lymphoblastic T cell leukaemia
NCIH209	0.001307	0.00148	0.186896	8.610089	lung	small cell carcinoma
JURKAT	0.001488	0.001655	0.117145	0.211954	haematopoietic and lymphoid tissue	acute lymphoblastic T cell leukaemia
NCIH1694	0.001753	0.000508	0.321443	21.59348	lung	small cell carcinoma
HCT15	0.001791	0.001473	0.183818	4.540784	large intestine	adenocarcinoma
JEKO1	0.001804	0.002309	0.117241	0.087378	haematopoietic and lymphoid tissue	mantle cell lymphoma
TALL1	0.001805	0.000895	0.199872	4.509292	haematopoietic and lymphoid tissue	acute lymphoblastic T cell leukaemia
A2780	0.002137	0.002099	0.181084	3.567055	ovary	adenocarcinoma
LOUCY	0.002474	0.002471	0.188038	2.522241	haematopoietic and lymphoid tissue	acute lymphoblastic T cell leukaemia
DLD1	0.002533	0.001467	0.226049	5.540107	large intestine	adenocarcinoma
U937	0.002554	0.002732	0.180761	1.946235	haematopoietic and lymphoid tissue	diffuse large B cell lymphoma
NCIH2081	0.002568	0.001459	0.304851	17.9809	lung	small cell carcinoma
TF1	0.002586	0.00158	0.288323	11.54969	haematopoietic and lymphoid tissue	acute myeloid leukaemia
RL	0.002993	0.001877	0.236617	1.95413	haematopoietic and lymphoid tissue	B cell lymphoma unspecified
NCIH2023	0.003031	0.002842	0.288394	13.34318	lung	adenocarcinoma
NAMALWA	0.003206	0.003265	0.24309	6.45672	haematopoietic and lymphoid tissue	Burkitt lymphoma
TE14	0.003393	0.002324	0.306836	12.67238	oesophagus	squamous cell carcinoma
KYSE510	0.003395	0.003418	0.199839	0.579743	oesophagus	squamous cell carcinoma
DMS79	0.003467	0.00128	0.384485	27.09377	lung	small cell carcinoma
NCIH2286	0.003508	0.00253	0.291713	11.19933	lung	small cell carcinoma
NCIH82	0.003543	0.003315	0.250228	4.909701	lung	small cell carcinoma
NCIH2087	0.003555	0.002583	0.382152	21.97574	lung	adenocarcinoma
PF382	0.003685	0.001894	0.276144	8.404679	haematopoietic and lymphoid tissue	acute lymphoblastic T cell leukaemia
YD38	0.003771	0.003547	0.298266	13.63878	upper aerodigestive tract	squamous cell carcinoma
HPAC	0.003928	0.002376	0.396472	20.84918	pancreas	ductal carcinoma
PECAPJ34CLONEC12	0.003966	0.003807	0.311032	11.80269	upper aerodigestive tract	squamous cell carcinoma

Cell line	*IC50(μM)*	*EC50(μM)*	*AUC*	*Emax*	*Tumor Type*	*Tumor Subtype*
MV411	0.004017	0.003916	0.216311	0.056467	haematopoietic and lymphoid tissue	acute myeloid leukaemia
AZ521	0.004189	0.003845	0.287487	8.891497	small intestine	NS
SNU16	0.004217	0.003693	0.282065	7.431244	stomach	undifferentiated adenocarcinoma
TE11	0.00428	0.003854	0.304041	10.15582	oesophagus	squamous cell carcinoma
NCIN87	0.004292	0.003935	0.313195	10.80159	stomach	NS
P31FUJ	0.004324	0.002434	0.285987	7.305396	haematopoietic and lymphoid tissue	acute myeloid leukaemia
CORL279	0.004345	0.004231	0.315531	11.3227	lung	small cell carcinoma
HUTU80	0.004361	0.004208	0.301592	8.150503	small intestine	adenocarcinoma
HCC95	0.0044	0.003342	0.407204	17.03582	lung	squamous cell carcinoma
RT11284	0.004435	0.00414	0.371749	18.19969	urinary tract	NS
G401	0.004449	0.004392	0.250793	3.80796	soft tissue	NS
K562	0.004556	0.004083	0.274996	4.382836	haematopoietic and lymphoid tissue	blast phase chronic myeloid leukaemia
MC116	0.004605	0.005215	0.252694	5.430331	haematopoietic and lymphoid tissue	B cell lymphoma unspecified
COLO320HSR	0.004609	0.00446	0.240303	0.900662	large intestine	adenocarcinoma
LNCAP	0.004648	0.003545	0.397386	21.5094	prostate	adenocarcinoma
NOMO1	0.005012	0.003922	0.329287	8.171852	haematopoietic and lymphoid tissue	acute myeloid leukaemia
KYSE150	0.005097	0.004407	0.305205	9.556776	oesophagus	squamous cell carcinoma
KYSE270	0.005351	0.005115	0.306237	7.792632	oesophagus	squamous cell carcinoma
C32	0.00536	0.005182	0.253167	1.007096	skin	NS
SKMEL28	0.005562	0.005627	0.279597	4.919564	skin	NS
WSUDLCL2	0.005671	0.004215	0.282886	0.634625	haematopoietic and lymphoid tissue	diffuse large B cell lymphoma
MKN1	0.005748	0.004335	0.39452	17.75546	stomach	mixed adenosquamous carcinoma
NCIH520	0.005895	0.005059	0.323681	10.58477	lung	squamous cell carcinoma
NCIH1836	0.006075	0.004881	0.333178	10.40721	lung	small cell carcinoma
CHL1	0.006077	0.004679	0.349333	13.21706	skin	NS
THP1	0.00615	0.004924	0.337139	12.47527	haematopoietic and lymphoid tissue	acute myeloid leukaemia
AGS	0.006186	0.005182	0.3536	14.1129	stomach	adenocarcinoma
TOV112D	0.006227	0.004264	0.3964	23.18819	ovary	endometrioid carcinoma
CAOV3	0.006468	0.005254	0.390842	19.3273	ovary	adenocarcinoma
KARPAS422	0.006525	0.005425	0.328997	8.084201	haematopoietic and lymphoid tissue	diffuse large B cell lymphoma
NCIH358	0.006571	0.003053	0.433186	22.65882	lung	bronchioloalveolar adenocarcinoma
TE6	0.006677	0.004833	0.362521	14.02411	oesophagus	squamous cell carcinoma
T.T	0.006752	0.004366	0.432105	24.08447	oesophagus	squamous cell carcinoma
NCIH524	0.006853	0.004977	0.361681	12.7766	lung	small cell carcinoma
TE9	0.00689	0.003749	0.424717	25.30919	oesophagus	squamous cell carcinoma
HSC4	0.006951	0.004332	0.43466	23.27648	upper aerodigestive tract	squamous cell carcinoma

Cell line	*IC50(μM)*	*EC50(μM)*	*AUC*	*Emax*	*Tumor Type*	*Tumor Subtype*
MIAPACA2	0.007021	0.005283	0.332162	9.710695	pancreas	ductal carcinoma
NCIH69	0.007089	0.00714	0.280211	1.270819	lung	small cell carcinoma
KM12	0.007345	0.006303	0.348209	12.23928	large intestine	adenocarcinoma
DV90	0.007364	0.005355	0.369777	11.78178	lung	adenocarcinoma
FTC133	0.007491	0.004279	0.414551	22.3385	thyroid	follicular carcinoma
NCIH1299	0.007498	0.005384	0.350839	12.64795	lung	non-small cell carcinoma
NUGC3	0.00772	0.005627	0.38244	15.99752	stomach	NS
NCIH1703	0.008047	0.004878	0.420393	21.7324	lung	adenocarcinoma
KYSE410	0.008253	0.005469	0.415601	21.4639	oesophagus	squamous cell carcinoma
NCIH2291	0.008405	0.007553	0.38223	13.48474	lung	adenocarcinoma
TE5	0.008472	0.006638	0.387656	15.07094	oesophagus	squamous cell carcinoma
U2OS	0.009007	0.007712	0.406496	9.064236	bone	NS
SW1463	0.009244	0.009177	0.340776	7.126189	large intestine	adenocarcinoma
HL60	0.009317	0.008115	0.370342	9.446155	haematopoietic and lymphoid tissue	acute myeloid leukaemia
NCIH716	0.009514	0.005771	0.379883	12.75198	large intestine	adenocarcinoma
NCIH661	0.009732	0.009063	0.360829	9.074949	lung	large cell carcinoma
SW480	0.009788	0.007726	0.409035	20.35763	large intestine	adenocarcinoma
BFTC905	0.009914	0.008929	0.39887	13.73926	urinary tract	transitional cell carcinoma
BT549	0.009983	0.005837	0.431039	20.54694	breast	ductal carcinoma
NCIH508	0.010235	0.009159	0.337789	3.207058	large intestine	adenocarcinoma
KU1919	0.010268	0.008773	0.395587	14.57044	urinary tract	transitional cell carcinoma
COLO741	0.01036	0.006858	0.418613	18.78304	skin	NS
MDAMB453	0.010402	0.008034	0.452361	21.95264	breast	NS
LU65	0.010583	0.00719	0.409074	11.99716	lung	non-small cell carcinoma
A375	0.010596	0.007623	0.440543	19.51092	skin	NS
HCC70	0.01099	0.008561	0.413475	13.84525	breast	ductal carcinoma
A101D	0.011003	0.006914	0.468887	20.45562	skin	NS
HLE	0.011279	0.010386	0.383928	7.875661	liver	hepatocellular carcinoma
RKO	0.011534	0.008496	0.382689	11.05361	large intestine	adenocarcinoma
NCIH460	0.011582	0.01108	0.371384	7.704677	lung	large cell carcinoma
SNB19	0.011607	0.007011	0.467719	20.73493	central nervous system	astrocytoma Grade IV
HUH1	0.011768	0.010591	0.414451	11.68072	liver	hepatocellular carcinoma
KMS11	0.012224	0.01094	0.391826	9.982711	haematopoietic and lymphoid tissue	plasma cell myeloma
647V	0.01224	0.01115	0.391223	10.17987	urinary tract	transitional cell carcinoma
TE4	0.012665	0.009106	0.394771	11.49063	oesophagus	squamous cell carcinoma
NCIH1437	0.013132	0.009476	0.408233	14.97097	lung	adenocarcinoma
HUH7	0.013955	0.012904	0.392615	7.054246	liver	hepatocellular carcinoma
143B	0.014366	0.013944	0.358228	2.195792	bone	NS
LS411N	0.016662	0.016827	0.385144	6.414977	large intestine	adenocarcinoma
SKMEL5	0.016833	0.014822	0.407428	8.227588	skin	NS
KYSE30	0.002426	10	0.44339	38.58605	oesophagus	squamous cell carcinoma

Cell line	*IC50(μM)*	*EC50(μM)*	*AUC*	*Emax*	*Tumor Type*	*Tumor Subtype*
CAKI1	0.004429	10	0.531935	34.02825	kidney	clear cell renal cell carcinoma
HCC1599	0.005645	0.003823	0.463913	32.70157	breast	ductal carcinoma
5637	0.006107	0.002239	0.485659	33.00819	urinary tract	NS
HCC1187	0.006507	0.003097	0.464968	33.99519	breast	ductal carcinoma
COLO680N	0.006972	0.004787	0.531063	33.76189	oesophagus	squamous cell carcinoma
SKBR3	0.007137	0.004323	0.439603	26.58705	breast	NS
NCIH1092	0.00716	0.003931	0.479044	26.40383	lung	small cell carcinoma
OCUM1	0.00779	10	0.532422	32.42435	stomach	diffuse adenocarcinoma
HSC2	0.007994	0.005088	0.451097	26.00789	upper aerodigestive tract	squamous cell carcinoma
NCIH1436	0.008754	10	0.495332	34.40202	lung	small cell carcinoma
SKMEL1	0.009242	0.000536	0.442658	22.0664	skin	NS
RD	0.009294	0.004792	0.470348	27.41572	soft tissue	embryonal
NCIH647	0.009841	0.006045	0.494182	28.28713	lung	mixed adenosquamous carcinoma
SCC4	0.010532	0.005568	0.461894	26.07786	upper aerodigestive tract	squamous cell carcinoma
NUGC4	0.010595	0.006625	0.464202	24.52784	stomach	signet ring adenocarcinoma
HMCB	0.011093	0.000815	0.504089	32.24977	skin	NS
COLO205	0.011489	0.005969	0.483296	27.13083	large intestine	adenocarcinoma
HEL	0.011746	0.003598	0.526792	28.88964	haematopoietic and lymphoid tissue	acute myeloid leukaemia
RPMI8226	0.01249	0.010401	0.436254	12.45893	haematopoietic and lymphoid tissue	plasma cell myeloma
SW780	0.013009	0.011796	0.449063	13.9761	urinary tract	transitional cell carcinoma
HCC1806	0.013019	0.005129	0.532503	36.86894	breast	ductal carcinoma
NCIH2347	0.01306	10	0.577758	40.28995	lung	adenocarcinoma
SHSY5Y	0.013453	0.00432	0.492725	25.03904	autonomic ganglia	NS
MKN45	0.013529	0.011004	0.442717	19.77129	stomach	diffuse adenocarcinoma
HS578T	0.013853	0.006902	0.537295	32.74703	breast	ductal carcinoma
HT1080	0.014147	0.01275	0.543789	29.73724	soft tissue	NS
HT55	0.014167	0.012668	0.470447	22.172	large intestine	adenocarcinoma
MESSA	0.014307	0.009003	0.493277	24.21537	soft tissue	NS
PC3	0.014471	0.011814	0.502021	26.1466	prostate	adenocarcinoma
U87MG	0.014695	10	0.514049	38.45109	central nervous system	astrocytoma
CALU6	0.014859	0.011452	0.459539	18.31843	lung	undifferentiated carcinoma
DAUDI	0.014888	0.013395	0.538295	25.4109	haematopoietic and lymphoid tissue	Burkitt lymphoma
KNS62	0.014901	0.012375	0.47611	18.83614	lung	squamous cell carcinoma
SCABER	0.015202	0.008931	0.568701	32.32311	urinary tract	transitional cell carcinoma
CFPAC1	0.015268	0.011256	0.52266	29.21979	pancreas	ductal carcinoma
NCIH1793	0.015288	0.008364	0.491588	27.47757	lung	non-small cell carcinoma
TE10	0.016273	0.009297	0.529321	29.64652	oesophagus	squamous cell carcinoma
NCIH1944	0.016307	10	0.580862	37.59181	lung	non-small cell carcinoma
DETROIT562	0.016685	0.010403	0.587348	36.50766	upper aerodigestive tract	NS

Cell line	*IC50(μM)*	*EC50(μM)*	*AUC*	*Emax*	*Tumor Type*	*Tumor Subtype*
NCIH522	0.01739	0.011534	0.444733	15.14236	lung	non-small cell carcinoma
GRANTA519	0.017643	0.01487	0.468567	17.90564	haematopoietic and lymphoid tissue	mantle cell lymphoma
DU145	0.01772	0.016588	0.432367	18.16451	prostate	NS
SNU398	0.017992	0.009627	0.49108	25.8616	liver	hepatocellular carcinoma
HLF	0.018027	0.011534	0.465709	18.24934	liver	hepatocellular carcinoma
NCIH1623	0.018862	0.00813	0.522025	30.06498	lung	adenocarcinoma
SNU668	0.01888	0.012849	0.464013	16.0231	stomach	signet ring adenocarcinoma
BXPC3	0.019388	0.008453	0.57381	36.5952	pancreas	ductal carcinoma
NCIH1930	0.020006	0.006616	0.540233	36.32772	lung	small cell carcinoma
HCT116	0.020431	0.004991	0.535649	35.91847	large intestine	NS
NCIH1355	0.020573	10	0.559364	35.37581	lung	adenocarcinoma
T84	0.020979	0.010096	0.492753	21.54238	large intestine	adenocarcinoma
DMS273	0.022219	0.016264	0.481488	16.57774	lung	small cell carcinoma
ECGI10	0.02244	10	0.568334	39.22919	oesophagus	NS
786O	0.022468	0.011656	0.504861	22.93315	kidney	clear cell renal cell carcinoma
HCC78	0.02392	0.012709	0.531509	23.40461	lung	adenocarcinoma
NCIH2122	0.023938	0.016742	0.501905	18.69937	lung	adenocarcinoma
OVISE	0.023953	10	0.540484	41.60625	ovary	clear cell carcinoma
LUDLU1	0.025828	0.012626	0.530668	28.03128	lung	squamous cell carcinoma
GP2D	0.026231	0.020627	0.506015	20.31771	large intestine	adenocarcinoma
WM2664	0.027533	10	0.583762	37.49186	skin	NS
PLCPRF5	0.02796	0.0164	0.510666	21.67484	liver	hepatocellular carcinoma
SW620	0.02926	0.026311	0.465758	9.079409	large intestine	adenocarcinoma
SKCO1	0.030585	10	0.595243	34.7288	large intestine	adenocarcinoma
A549	0.032375	0.018492	0.573044	31.10718	lung	non-small cell carcinoma
NCIH929	0.034475	0.020579	0.52854	24.32478	haematopoietic and lymphoid tissue	plasma cell myeloma
ACHN	0.034708	0.030334	0.453014	2.196602	kidney	renal cell carcinoma
NCIH1975	0.034766	0.025525	0.528991	17.89159	lung	non-small cell carcinoma
PANC0213	0.035795	0.021461	0.582932	32.945	pancreas	NS
HT1376	0.036268	0.017852	0.551857	18.92436	urinary tract	transitional cell carcinoma
HT29	0.037031	0.022837	0.574146	29.83252	large intestine	adenocarcinoma
UMUC3	0.039504	0.021864	0.593428	35.04699	urinary tract	transitional cell carcinoma
SNU761	0.039689	10	0.581426	38.06756	liver	hepatocellular carcinoma
SKLU1	0.040003	0.008488	0.56624	38.28472	lung	adenocarcinoma
HCC1954	0.040563	10	0.591175	43.62567	breast	ductal carcinoma
NCIH23	0.042711	0.037736	0.622699	27.39399	lung	non-small cell carcinoma
KATOIII	0.043772	0.040387	0.490001	12.87902	stomach	adenocarcinoma
A2058	0.046764	0.028087	0.56942	30.63873	skin	NS
SKMES1	0.047461	0.024323	0.60692	30.65752	lung	squamous cell carcinoma
SW48	0.050006	10	0.603764	37.9499	large intestine	adenocarcinoma
HEPG2	0.052079	10	0.608647	37.75892	liver	hepatocellular carcinoma

Cell line	*IC50(μM)*	*EC50(μM)*	*AUC*	*Emax*	*Tumor Type*	*Tumor Subtype*
SNUC2A	0.052512	0.024542	0.615038	34.41705	large intestine	adenocarcinoma
LOVO	0.057826	10	0.636191	47.95459	large intestine	adenocarcinoma
HSC3	0.060326	10	0.6313	47.25644	upper aerodigestive tract	squamous cell carcinoma
JIMT1	0.061923	0.046579	0.551921	13.45549	breast	ductal carcinoma
SW1710	0.063291	0.027254	0.5768	25.48455	urinary tract	transitional cell carcinoma
RERFLCMS	0.065898	0.023477	0.641498	38.15621	lung	non-small cell carcinoma
HS294T	0.066791	0.030717	0.600533	28.13572	skin	NS
ASPC1	0.066825	0.018855	0.581226	32.80606	pancreas	ductal carcinoma
PANC1	0.06858	0.051468	0.554273	12.02616	pancreas	ductal carcinoma
CAPAN1	0.115609	10	0.639142	40.2242	pancreas	ductal carcinoma
NCIH446	0.119463	0.014536	0.583736	24.17448	lung	small cell carcinoma
HEP3B217	0.129298	10	0.644762	47.20301	liver	hepatocellular carcinoma
NCIH2172	0.132833	10	0.68115	37.97151	lung	non-small cell carcinoma
SKMEL2	0.13968	0.026999	0.639177	35.37217	skin	NS
SNU5	0.146396	0.082367	0.683652	32.15273	stomach	undifferentiated adenocarcinoma
NCIH2228	0.178529	10	0.686403	44.26922	lung	adenocarcinoma
BICR18	0.19427	0.186872	0.618995	4.76155	upper aerodigestive tract	squamous cell carcinoma
GI1	0.28744	0.295062	0.623711	9.190768	central nervous system	gliosarcoma
MCF7	0.434714	10	0.659214	43.69748	breast	NS
SKMEL3	0.472006	10	0.620722	46.89633	skin	NS
WM115	0.477738	0.002502	0.609719	36.92872	skin	NS
COLO679	2.044655	2.344991	0.502041	24.71872	skin	NS
SNGM	4.901703	4.138108	0.916457	26.87739	endometrium	adenocarcinoma
NCIH2171	10	10	0.7416	16.52996	lung	small cell carcinoma
SUPT1	3.3333	10	0.691296	48.11215	haematopoietic and lymphoid tissue	acute lymphoblastic T cell leukaemia
KNS81	10	10	0.630939	58.5801	central nervous system	astrocytoma Grade IV
MPP89	10	10	0.638426	51.42635	pleura	NS
SNU886	10	10	0.642533	48.34561	liver	hepatocellular carcinoma
COLO829	10	10	0.655706	47.96395	skin	NS
KALS1	10	10	0.65593	49.78685	central nervous system	NS
SNU878	10	10	0.664801	52.14968	liver	hepatocellular carcinoma
GAK	10	10	0.664855	47.69716	NOT IN CCLE	NOT IN CCLE
OSRC2	10	10	0.665792	52.3524	kidney	renal cell carcinoma
RCM1	10	10	0.671054	42.54585	large intestine	adenocarcinoma
NCIH1734	10	10	0.673415	54.90475	lung	adenocarcinoma
DMS153	10	10	0.673713	50.81473	lung	small cell carcinoma
J82	10	10	0.674852	48.70006	urinary tract	transitional cell carcinoma
BICR22	10	10	0.682919	54.75925	upper aerodigestive tract	squamous cell carcinoma
SW1990	10	10	0.689389	49.22702	pancreas	ductal carcinoma
HCC1143	10	10	0.69059	50.38194	breast	ductal carcinoma
HCC2935	10	10	0.692215	50.08429	lung	non-small cell carcinoma

Cell line	*IC50(μM)*	*EC50(μM)*	*AUC*	*Emax*	*Tumor Type*	*Tumor Subtype*
KYSE70	10	10	0.694176	50.18949	oesophagus	squamous cell carcinoma
MDAMB468	10	10	0.697183	50.86583	breast	NS
NMCG1	10	10	0.701293	47.05987	central nervous system	NS
SKHEP1	10	10	0.70906	44.3029	liver	adenocarcinoma
SW837	10	10	0.710855	46.73384	large intestine	adenocarcinoma
A204	10	10	0.713626	58.90593	soft tissue	NS
HCC1419	10	10	0.720097	52.41521	breast	ductal carcinoma
BT474	10	10	0.720573	53.87865	breast	ductal carcinoma
MEWO	10	10	0.723674	56.56317	skin	NS
MDAMB231	10	10	0.726163	55.94067	breast	NS
CALU3	10	10	0.727683	50.88262	lung	adenocarcinoma
SKNSH	10	10	0.729486	53.89154	autonomic ganglia	NS
ONS76	10	10	0.73362	54.63371	central nervous system	NS
MDAMB175VII	10	10	0.738455	60.66071	breast	ductal carcinoma
SKMEL31	10	10	0.739322	59.48678	skin	NS
DMS53	10	10	0.742606	49.07034	lung	small cell carcinoma
SNUC1	10	10	0.747771	55.45382	large intestine	adenocarcinoma
NCIH1573	10	10	0.748221	53.57475	lung	adenocarcinoma
NCIH1435	10	10	0.761242	60.04075	lung	non-small cell carcinoma
NIHOVCAR3	10	10	0.777131	59.47137	ovary	NS
SCC25	10	10	0.778864	62.3079	upper aerodigestive tract	squamous cell carcinoma
NCIH441	10	10	0.78042	67.48763	lung	adenocarcinoma
HCC1428	10	10	0.781839	59.9889	breast	NS
VCAP	10	10	0.782184	62.59825	prostate	adenocarcinoma
NCIH1838	10	10	0.785788	60.0585	lung	non-small cell carcinoma
SKMEL24	10	10	0.7882	58.11712	skin	NS
LU99	10	10	0.78999	64.65551	lung	large cell carcinoma
NCIH2009	10	8.019371	0.791426	55.69455	lung	adenocarcinoma
RPMI7951	10	10	0.806669	66.30924	skin	NS
NCIH596	10	10	0.815117	61.33612	lung	mixed adenosquamous carcinoma
TE8	10	10	0.816021	72.1307	oesophagus	squamous cell carcinoma
SW1271	10	10	0.817932	65.37809	lung	small cell carcinoma
C2BBE1	10	10	0.830329	59.34028	large intestine	adenocarcinoma
CAL27	10	10	0.833497	74.67924	upper aerodigestive tract	squamous cell carcinoma
MDAMB157	10	10	0.836176	64.71391	breast	ductal carcinoma
UACC812	10	6.947929	0.841822	62.67078	breast	ductal carcinoma
MDAMB436	10	10	0.843786	70.42433	breast	NS
NCIH1395	10	10	0.854187	70.68192	lung	adenocarcinoma
HS739T	10	10	0.868583	78.34986	breast	NS
KURAMOCHI	10	10	0.874218	73.84661	ovary	undifferentiated carcinoma
NCIH1650	10	10	0.874566	68.07063	lung	bronchioloalveolar adenocarcinoma

Cell line	*IC50(μM)*	*EC50(μM)*	*AUC*	*Emax*	*Tumor Type*	*Tumor Subtype*
CCFSTTG1	10	10	0.879034	78.89955	central nervous system	astrocytoma
CAPAN2	10	10	0.880831	75.01844	pancreas	ductal carcinoma
HCC827	10	10	0.881358	74.84	lung	adenocarcinoma
KASUMI1	10	10	0.882289	81.08059	haematopoietic and lymphoid tissue	acute myeloid leukaemia
HS852T	10	10	0.883001	78.51343	skin	NS
HT144	10	10	0.89125	81.60806	skin	NS
HCC1937	10	10	0.9094	80.9493	breast	ductal carcinoma
BT20	10	10	0.911675	78.79204	breast	ductal carcinoma
SW579	10	10	0.920274	79.98259	thyroid	anaplastic carcinoma
HS695T	10	10	0.923787	74.11092	skin	NS
T24	10	10	0.928907	75.74279	urinary tract	transitional cell carcinoma
TE1	10	10	0.937963	84.54246	oesophagus	squamous cell carcinoma
MDAMB415	10	10	0.941323	83.39028	breast	NS
SCC9	10	10	0.942441	69.24641	upper aerodigestive tract	squamous cell carcinoma
SCC15	10	10	0.948945	90.39119	upper aerodigestive tract	squamous cell carcinoma
KG1	10	10	0.959161	80.43661	haematopoietic and lymphoid tissue	acute myeloid leukaemia
SW1417	10	10	0.963372	91.96064	large intestine	adenocarcinoma
NCIH1563	10	10	0.965176	93.507	lung	adenocarcinoma
DAOY	10	10	0.968421	83.40166	central nervous system	NS
HUH28	10	10	0.98625	92.15381	biliary tract	NS
HCC38	10	10	0.98639	93.61212	breast	ductal carcinoma
MALME3M	10	10	0.986698	94.08447	skin	NS
SKOV3	10	10	0.990095	96.03318	ovary	adenocarcinoma
G361	10	10	1.043507	89.52446	skin	NS
SAOS2	10	10	1.080519	92.52133	bone	NS
TE15	10	10	1.084416	89.04183	oesophagus	squamous cell carcinoma
HS839T	10	10	1.09737	101.2434	skin	NS
HS688AT	10	10	1.105351	98.46712	skin	NS
MDAMB361	10	10	1.116565	94.55241	breast	NS
CALU1	10	10	1.217208	118.4694	lung	squamous cell carcinoma
NCIH1755	10	10	1.289838	123.3293	lung	adenocarcinoma

Example 3: Determination of Anti-Proliferative Activity in a Xenograph Model

[00130] The therapeutic in vivo efficacy of Compound 1 was evaluated in SU60 colorectal cancer, KYSE-150 esophageal cancer, COH70 triple-negative breast cancer, GXA-3036 gastric adenocarcinoma and OCI-LY19 Diffuse Large B-Cell Non-Hodgkin's Lymphoma xenograft models. In all studies, efficacy was determined based on percent tumor growth inhibition (TGI), differences in mean tumor growth, and statistical assessment of differences in net tumor volume

distributions on the day of TGI analysis between control and treated animals. Tolerability was assessed based on differences in mean body weights between control and treated animals over the course of the study.

A. In Vivo Efficacy of Compound 1 in the SU60 Patient-Derived Colorectal Cancer Xenograft Model

[00131] The *in vivo* therapeutic efficacy and tolerability of Compound 1, administered orally as the free acid form in a vehicle comprised of 50% polyethylene glycol 400 (PEG 400) and 50% PBS (pH 9), was evaluated in the SU60 CRC PDX model established in female non-obese diabetic/severe combined immunodeficiency (NOD/SCID) gamma (NSG) mice. Each mouse was inoculated subcutaneously in the abdomen with 2 × 106 SU60 tumor cells (0.1 ml) suspended in Matrigel. When the average tumor size reached ~270 mm3, test animals were randomized into 6 groups consisting of 8 mice per group and treatments were initiated. Control mice received 50% PEG 400 and 50% PBS (pH 9) vehicle PO QD x7. Treated mice received Compound 1 in vehicle PO at 20 or 10 mg/kg QD x7, at 20 mg/kg QOD, at 23 mg/kg 3 on / 4 off, or at 14 mg/kg at 5 on / 2 off. TGI was determined on Day 19 as (1 – Diff treated/Diff control) x 100. The difference in mean net tumor volumes on the day of TGI analysis for treated versus control animals was evaluated statistically using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. Adjusted p-values are shown where a calculated probability (p) ≤ 0.05 was considered statistically significant. Individual tumors were measured twice weekly in two dimensions using a caliper, and the tumor volumes (TV) in mm3 were calculated using the formula: $TV = 0.5\ (l \times w \times h)$, where l, w, and h are the tumor length, width, and height in mm, respectively; Mean tumor volumes ± standard error of the mean (SEM) were calculated and plotted versus days of dosing (Fig. 10 left panel). Body weights were measured twice weekly. Body weights ± SEM were calculated and plotted versus days of dosing (Fig. 10 right panel).

[00132] As presented in Table 4, all five Compound 1 regimens were significantly efficacious ($p < 0.005$) based on the statistical assessment of differences in mean net tumor volumes on the day of TGI analysis for treated versus control animals. Compound 1 at 20 or 10 mg/kg dosed once daily for 7 days (QD x7) yielded dose-dependent TGIs of 71% and 48%. Four Compound 1 dosing regimens: QD x7, once every other day (QOD), once daily for 3 days followed by no dosing for 4 days repeated to study end (3 on / 4 off), and 5 on / 2 off were tested at similar weekly dose levels (69 to 70 mg/kg/wk) to identify an optimal intermittent dosing schedule. The intermittent schedules of 3 on / 4 off and 5 on / 2 off delivered similar tumor

growth responses as QD x7 (52% and 42% vs. 48% TGI). Compound 1 at 20 mg/kg administered QOD was the least efficacious regimen yielding 29% TGI.

[00133] As shown in Fig. 10 (left panel), tumor growth in the Compound 1-treated groups was reduced relative to control animals. The relative rates of tumor growth among the various Compound 1-treated groups were consistent with the TGI attained at study end. As presented in Fig. 10 (right panel), Compound 1 appeared well-tolerated with nominal mean body weight changes over the course of the study.

Table 4

Treatment Regimen						Median Tumor Volumes (mm^3)				Statistical Significance (p-value)
n	Agent	Dose (mg/kg) Single	Dose (mg/kg) Weekly	Route	Schedule	Day 1	Day 19	Diff.	%TGI	
8	Vehicle	-	-	PO	QD x7	266	2011	1745	-	-
8	Compd 1	20	140	PO	QD x7	261	767	506	71	< 0.0001
8	Compd 1	10	70	PO	QD x7	250	1151	901	48	< 0.0001
8	Compd 1	20	70	PO	QOD	285	1527	1242	29	0.0049
8	Compd 1	23	69	PO	3 on / 4 off	288	1121	833	52	< 0.0001
8	Compd 1	14	70	PO	5 on / 2 off	281	1300	1019	42	< 0.0001

B. *In Vivo* Efficacy of Compound 1 in the KYSE-150 Human Esophageal Cancer Cell Line Xenograft Model

[00134] Study #1 - The in vivo therapeutic efficacy and tolerability of Compound 1, administered orally as the lysine salt suspended in a vehicle comprised of 10% PEG 400 and 90% of 0.5% methylcellulose (MC), was evaluated in the KYSE-150 ESCC CDX model established in female NSG mice. As presented in Table 5, compound 4 at 5, 15, or 50 mg/kg (free acid equivalents) administered on the 3 on / 4 off schedule yielded dose-dependent TGIs of 19%, 50%, and 72%, respectively. Differences in mean net tumor volumes on the day of TGI analysis for treated versus control animals were significant for Compound 1 at 15 and 50 mg/kg ($p < 0.001$) but not at 5 mg/kg ($p > 0.05$). As shown in Fig. 11 (left panel), tumor growth in the Compound 1-treated groups was reduced relative to control animals. The relative rates of tumor growth among the various Compound 1- treated groups were consistent with the TGI attained at study end. As presented in Fig. 11 (right panel), Compound 1 appeared well-tolerated with nominal mean body weight gains over the course of the study. Each mouse was inoculated subcutaneously in the right flank with1 × 107 KYSE-150 tumor cells (0.1 ml) suspended in PBS. When the average tumor size reached ~117 mm3, test animals were randomized into 4 groups consisting of 9 mice per group and treatments were initiated. Control mice received 10% PEG 400 and 90% of 0.5% MC vehicle PO 3 on / 4 off. Treated mice received the Compound 1 lysine salt form suspended in vehicle at 5, 15, or 50 mg/kg on the same schedule. TGI was

determined on Day 19 as (1 - Difftreated/Diffcontrol) x 100; The difference mean net tumor volumes on the day of TGI analysis for treated versus control animals was evaluated statistically using one-way ANOVA followed by Dunnett's multiple comparisons test; Adjusted p-values are shown where a calculated probability (p) ≤ 0.05 was considered statistically significant. Individual tumors were measured twice weekly in three dimensions using a caliper, and the tumor volumes (TV) in mm3 were calculated using the formula: TV = 0.5 (l × w × h), where l, w, and h are the tumor length, width, and height in mm, respectively; Mean tumor volumes ± SEM were calculated and plotted versus days of dosing (left panel). Body weights were measured twice weekly. Body weights ± SEM were calculated and plotted versus days of dosing (right panel).

Table 5

n	Treatment Regimen				Median Tumor Volumes (mm^3)				Statistical Significance (p-value)
	Agent	Dose (mg/kg)	Route	Schedule	Day 1	Day 19	Diff.	%TGI	
9	Vehicle	-	PO	3 on/4 off	104	988	884	--	--
9	Compound 1	5	PO	3 on/4 off	105	824	719	19%	0.0538
9	Compound 1	15	PO	3 on/4 off	127	569	442	50%	0.0006
9	Compound 1	50	PO	3 on/4 off	109	359	250	72%	< 0.0001

[00135] Study #2 - The in vivo therapeutic efficacy and tolerability of Compound 1, administered orally as the lysine salt suspended in a vehicle comprised of 10% PEG 400 and 90% of 0.5% MC, was evaluated in the KYSE-150 ESCC CDX model established in female NOD/SCID mice. Each mouse was inoculated subcutaneously in the right flank with 1 × 107 KYSE-150 tumor cells (0.1 ml) suspended in 50% Matrigel. When the average tumor size reached ~153 mm3, test animals were randomized into 4 groups consisting of 8 mice per group and treatments were initiated. Control mice received 10% PEG 400 and 90% of 0.5% MC vehicle PO QDx21. Treated mice received the Compound 1 lysine salt form suspended in vehicle at 10, 15, or 20 mg/kg on the same schedule. TGI was determined on Day 21 as (1 - Difftreated/Diffcontrol) x 100. Individual tumors were measured twice weekly in two dimensions using a caliper, and the tumor volumes (TV) in mm3 were calculated using the formula: TV = 0.5 a × b2, where a and b are the long and short tumor diameters in mm, respectively; Mean tumor volumes ± SEM were calculated and plotted versus days of dosing (Fig. 12 left panel). Body weights were measured twice weekly. Body weights ± SEM were calculated and plotted versus days of dosing (Fig. 12 right panel).

[00136] The difference in mean net tumor volumes on the day of TGI analysis for treated versus control animals was evaluated statistically using one-way ANOVA followed by Dunnett's

multiple comparisons test. Adjusted p-values are shown where a calculated probability (p) ≤ 0.05 was considered statistically significant.

[00137] As presented in Table 6, Compound 1 at 10, 15, or 20 mg/kg (free acid equivalents) administered on the QD x21 schedule yielded dose-dependent TGIs of 54%, 76%, and 84%, respectively. Differences in mean net tumor volumes on the day of TGI analysis for treated versus control animals were significant ($p < 0.02$).

[00138] As shown in Fig. 12 (left panel), tumor growth in the Compound 1-treated groups was reduced relative to control animals. The relative rates of tumor growth among the various Compound 1- treated groups were consistent with the TGI attained at study end. As presented in Fig. 12 (right panel), Compound 1-treated animals did not experience the body weight gains exhibited by control animals but appeared well-tolerated with nominal mean body weight changes over the course of the study.

Table 6

n	Treatment Regimen				Median Tumor Volumes (mm^3)				Statistical Significance (p-value)
	Agent	Dose (mg/kg)	Route	Schedule	Day 1	Day 21	Diff.	%TGI	
8	Vehicle	-	PO	QD x 21	153	2076	1923	--	--
8	Compound 1	10	PO	QD x 21	151	1027	876	54	0.0155
8	Compound 1	15	PO	QD x 21	152	620	468	76	0.0002
8	Compound 1	20	PO	QD x 21	154	469	315	84	< 0.0001

C. In Vivo Efficacy of Compound 1 in the COH70 Patient-Derived Triple Negative Breast Cancer Xenograft Model

[00139] The in vivo therapeutic efficacy and tolerability of Compound 1, administered orally as the free acid form in a PBS vehicle plus two equivalents of NaOH (final pH 10), was evaluated in a dose range study using the COH70 TNBC PDX model established in female NSG mice. Each mouse was inoculated subcutaneously in the abdomen with 2 × 106 COH70 tumor cells (0.2 ml) suspended in Matrigel. When the average tumor size reached ~107 mm3, test animals were randomized into 5 groups consisting of 9 mice per group and treatments were initiated. Control mice received PBS (pH 10) vehicle PO QDx36. Treated mice received Compound 1 (dissolved in PBS + 2 equivalents of NaOH, final pH 10) PO at 50, 40, 25, or 12.5 mg/kg QDx36. TGI was determined on Day 36 as (1 - Difftreated/Diffcontrol) x 100. Individual tumors were measured twice weekly in two dimensions using a caliper, and the tumor volumes (TV) in mm3 were calculated using the formula: TV = 0.5 (l × w × h), where l, w, and h are the tumor length, width, and height in mm, respectively. Mean tumor volumes ± SEM were

calculated and plotted versus days of dosing (left panel). Body weights were measured twice weekly. Body weights ± SEM were calculated and plotted versus days of dosing (right panel).

[00140] The difference in the distribution of net tumor volumes on the day of TGI analysis for treated versus control animals was evaluated statistically using one-way ANOVA followed by Dunnett's multiple comparisons test; Adjusted p-values are shown where a calculated probability (p) ≤ 0.05 was considered statistically significant; These are tests of statistical significance and do not provide an estimate of the size of the difference between groups nor are they a measure of clinical or biological significance.

[00141] As presented in Table 7, Compound 1 at 12.5, 20, 40, or 50 mg/kg administered on the QD x36 schedule was significantly efficacious ($p < 0.0001$), based on differences in mean net tumor volumes on the day of TGI analysis for treated versus control animals, yielding respective TGIs of 57%, 68%, 86%, and 86%. Compound 1 yielded dose-dependent inhibition of tumor growth that attained a plateau at doses ≥40 mg/kg.

[00142] As shown in Fig. 13 (left panel), tumor growth in the Compound 1-treated groups was reduced relative to control animals. The relative rates of tumor growth among the various Compound 1- treated groups were consistent with the TGI attained at study end. As presented in Fig. 13 (right panel), Compound 1 appeared well-tolerated with nominal dose-dependent mean body weight changes (≤6.5% mean body weight loss) over the course of the study.

Table 7

Treatment Regimen					Median Tumor Volumes (mm^3)				Statistical Significance (p-value)
n	Agent	Dose (mg/kg)	Route	Schedule	Day 1	Day 36	Diff.	%TGI	
9	Vehicle	-	PO	QD x36	110	1455	1346	--	--
9	Compound 1	12.5	PO	QD x36	104	685	581	57	< 0.0001
9	Compound 1	25	PO	QD x36	99	524	424	68	< 0.0001
9	Compound 1	40	PO	QD x36	98	282	184	86	< 0.0001
9	Compound 1	50	PO	QD x36	103	295	191	86	< 0.0001

D. In Vivo Efficacy of Compound 1 in the GXA-3036 Gastric Adenocarcinoma Patient-Derived Xenograft Model

[00143] The in vivo efficacy and tolerability of Compound 1, administered orally as the lysine salt form suspended in a vehicle comprised of 5% 2-hydroxypropyl-beta-cyclodextrin (HPBCD) in 50 mM phosphate buffer (pH 7.4), were evaluated in female immunodeficient NMRI-Foxn1nu mice using the subcutaneous gastric adenocarcinoma GXA-3036, a moderately differentiated intestinal type (based on Lauren's criteria) PDX model of Asian origin. Each mouse was inoculated subcutaneously in the right flank with a GXA-3036 tumor fragment (3 to

4 mm edge length). When the average tumor size reached ~109 mm3, test animals were randomized into 5 groups consisting of 8 mice per group and treatments were initiated. Control mice received 5% HPBCD in 50 mM phosphate buffer (pH 7.4) vehicle PO 3 on / 4 off. Treated mice received the Compound 1 lysine salt form in vehicle at 5, 15, or 50 mg/kg on the same schedule or at 22.5 mg/kg 2 on / 5 off. One control animal, classified as moribund, was euthanized on Day 17 and was censored from analysis. Individual tumors were measured twice weekly in two dimensions using a caliper, and the tumor volumes (TV) in mm3 were calculated using the formula: TV = 0.5 a × b2, where a and b are the long and short diameters in mm, respectively. Mean tumor volumes ± SEM were calculated and plotted versus days of dosing (Fig. 14 left panel). Body weights were measured twice weekly. Body weights ± SEM were calculated and plotted versus days of dosing (Fig. 14 right panel).

[00144] TGI was determined on Days 35 and 38 as (1 - Difftreated/Diffcontrol) x 100. The differences in median net tumor volumes on the days of TGI analysis for treated versus control animals were evaluated statistically using the non-parametric Kruskal-Wallis test followed by Dunn's multiple comparisons test. The reported adjusted p-values are for both days of TGI analysis where a calculated probability (p) ≤ 0.05 was considered statistically significant.

[00145] As presented in Table 8, Compound 1 at 5, 15, or 50 mg/kg (free acid equivalents) administered on the 3 on / 4 off schedule yielded respective TGIs of 52%, 71%, and 55% on Day 35 and 43%, 69%, and 52% on Day 38. The lower TGI exhibited by the 5 mg/kg Compound 1 group on D38 was due to a moderate increase in tumor volume for a single animal that shifted the median. Compound 1 at 22.5 mg/kg (free acid equivalents) administered on the 2 on / 5 off schedule yielded respective Day 35 and Day 38 TGIs of 42% and 41%. Differences in median net tumor volumes on both days of TGI analyses for treated versus control animals were significant for Compound 1 at 15 mg/kg ($p = 0.0007$) but not for the other treatment regimens ($p > 0.05$).

[00146] As shown in Fig. 14 (left panel), tumor growth in the Compound 1-treated groups was reduced relative to control animals. The relative rates of tumor growth among the various Compound 1- treated groups were consistent with the TGI attained at study end. As presented in Fig. 14 (right panel), Compound 1 appeared well-tolerated with little change in mean body weights over the course of the study.

Table 8

Group	n	Treatment Regimen				Median Tumor Volume (mm^3)							Statistical Significance (p-value)
		Agent	Dose (mg/kg)	Route	Schedule	Day 0	Day 35	Diff.	%TGI	Day 38	Diff.	%TGI	
1	7	Vehicle	-	PO	3 on / 4 off	111	698	587	--	728	617	--	---
2	8	Compound 1	5	PO	3 on / 4 off	109	391	282	52%	460	351	43%	>0.05
3	8	Compound 1	15	PO	3 on / 4 off	109	278	169	71%	298	189	69%	0.0007
4	8	Compound 1	50	PO	3 on / 4 off	109	371	262	55%	403	294	52%	>0.05
5	8	Compound 1	22.5	PO	*2 on / 5 off	109	447	338	42%	470	361	41%	>0.05

E. In Vivo Efficacy of Compound 1 in the OCI-LY19 Human Diffuse Large B-Cell Non-Hodgkin's Lymphoma Xenograft Model

[00147] The in vivo efficacy and tolerability of Compound 1, administered orally as the lysine salt form suspended in a 0.5% MC vehicle, were evaluated in female C.B-17 severe combined immunodeficient (scid) mice inoculated subcutaneously with OCI-LY19 tumor cells established from the bone marrow of a female patient with diffuse large B-cell non-Hodgkin's lymphoma (DLBCL), stage 4B, at relapse. Each mouse was inoculated subcutaneously in the right flank with 5 × 106 OCI-LY19 tumor cells (0.1 ml) suspended in 50% Matrigel. When the average tumor size reached ~165 mm3, test animals were randomized into 6 groups consisting of 8 mice per group and treatments were initiated. Study control mice received 0.5% MC vehicle PO QD 3 on / 4 off and positive control mice received CHOP therapy QDx5. Compound 1-treated mice received the Compound 1 lysine salt form suspended in vehicle at 5, 15, or 50 mg/kg QD or at 25 mg/kg BID on the 3 on / 4 off schedule. TGI was determined on Day 21 as (1 - Difftreated/Diffcontrol) x 100. Individual tumors were measured on the days shown in two dimensions using a caliper, and the tumor volumes (TV) in mm3 were calculated using the formula: TV = 0.5 a × b2, where a and b are the long and short tumor diameters in mm, respectively. Mean tumor volumes ± SEM were calculated and plotted versus days of dosing (Fig. 15 left panel). Body weights were measured twice weekly. Body weights ± SEM were calculated and plotted versus days of dosing (Fig. 15 right panel). The difference in mean net tumor volumes on the day of TGI analysis for treated versus control animals was evaluated statistically using one-way ANOVA followed by Dunnett's multiple comparisons test; Adjusted p-values are shown where a calculated probability ($p) \leq 0.05$ was considered statistically significant.

[00148] As presented in Table 9, Compound 1 at 5, 15, or 50 mg/kg administered QD or at 25 mg/kg (free acid equivalents) administered twice daily (BID) on the 3 on / 4 off schedule yielded respective dose-dependent TGIs of 55%, 83%, 90%, and 102% on Day 21. As shown in

Fig. 15, differences in mean net tumor volumes on the day of TGI analysis for Compound 1-treated versus control animals were significant ($p < 0.0001$).

[00149] The gold standard first line chemotherapy regimen CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) recommended in the treatment of non-Hodgkin's lymphoma was included as a positive control. CHOP administered QD x5 yielded a TGI of 108% on Day 21 and appeared curative in seven of eight test animals that exhibited no measurable tumor volume for eight or more consecutive days.

[00150] As shown in Fig. 15 (left panel), tumor growth in the Compound 1-treated groups was reduced relative to control animals. The relative rates of tumor growth among the various Compound 1- treated groups were consistent with the TGI attained at study end. As presented in Fig. 15 (right panel), compound 1 appeared well-tolerated with little change in mean body weights over the course of the study.

Table 9

Group	n	Treatment Regimen				Median Tumor Volume (mm^3)				Statistical Significance (p-value)
		Agent	Dose (mg/kg)	Route	Schedule	Day 0	Day 21	Diff.	%TGI	
1	8	Vehicle	-	PO	QD 3 on/4 off	167	2227	2061	--	---
2	8	cyclophosphamide	5	IV	QD x5	164	0	-164	108%	< 0.0001
		doxorubicin	0.5	IV						
		vincristine	0.08	IV						
		prednisolone	0.03	PO						
3	8	Compound 1	5	PO	QD 3 on/4 off	168	1088	920	55%	< 0.0001
4	8	Compound 1	15	PO	QD 3 on/4 off	167	512	345	83%	< 0.0001
5	8	Compound 1	50	PO	QD 3 on/4 off	165	361	196	90%	< 0.0001
6	8	Compound 1	25	PO	BID 3 on/4 off	167	135	-32	102%	< 0.0001

Example 4: Determination of Cancer Stem Cell Characteristics Following Treatment with Compound 1

[00151] The ability of Compound 1 to affect tumorigenic potential was analyzed by flow cytometry analysis of the tumorigenic cell population and functional in vivo tumorigenicity assay (limiting dilution assay).

[00152] To assess the effect of Compound 1 on tumorigenic cell population, Compound 1 was used to treat SU60 xenograft tumors. NSG female mice engrafted with SU60 cells (7 mice each group) were administered Compound 1 at 40 mg/kg PO QD x21. The day after the last dose, three tumors nearest to the mean size from each group were dissociated and the single cells were used to perform: 1) flow cytometry analysis of the tumorigenic cell population and 2) functional in vivo tumorigenicity assay (limiting dilution assay).

Flow Cytometry Analysis of Tumorigenic Cell Population after Compound 1 Treatment

[00153] The proportion of tumorigenic $CD44^{High};EpCAM^{+}$ cells in the cell population was quantified by flow cytometry. Compound 1 significantly reduced by 2.5 fold the proportion of tumorigenic cells ($CD44^{High};EpCAM^{+}$) compared to vehicle control, see Fig. 16. Flow cytometry analysis performed on single cell isolated from 3 vehicle-treated and 3 Compound 1-treated tumors. P-value was determined with unpaired Student T-test.

Functional In Vivo Tumorigenicity Assay After Compound 1 Treatment

[00154] To study the effect of Compound 1 on tumor initiating cell (TIC) frequency in CRC tumors, we evaluated the tumorigenic potential of SU60 xenograft cells post treatment with Compound 1 or vehicle. At the end of dosing, SU60 dissociated cells from three animals per group nearest to the mean size were injected in a limiting dilution manner into recipient NSG mice and tumor volumes were measured. The number of cells required for tumor formation was reduced in the Compound 1-treated group. The reduction in TIC frequency was 4.4-fold (Fig. 17 and Table 10). Differentiation of progenitor cells is a potential explanation for this reduction.

Table 10

Treatment Group	Tumor Take Rate & Mean Tumor Volume (1000 cells)	Tumor Take Rate & Mean Tumor volume (500 cells)	Tumor Take Rate & Mean Tumor Volume (50 cells)	TIC frequency
Vehicle	10/10 1588 mm^3	8/10 1290 mm^3	8/10 984 mm^3	1 in 144 cells
Compound 1, 40 mg/kg, QD	7/10 830 mm^3	5/10 589 mm^3	3/10 209 mm^3	1 in 643 cells

Example 5: Sensitivity of various colorectal cancer cells lines with MSI-H status to Compound 1 treatment

[00155] To further study the efficacy of Compound 1, the sensitivity of various colon cancer cell lines with certain genes of interest to Compound 1 was evaluated. These certain genes of interest are found in frequently mutated pathways such as gene mismatch repair, beta-catenin, MAPK, PI3K, p53, TGFbeta, chromatin remodeling, and histone modification pathways. Fig. 18 shows a heatmap of the mutation status of genes in these pathways along with the respective Microsatellite instability (MSI-H), CpG island methylator phenotype (CIMP), and MLH1 methylation status, in which the cell lines are sorted by their sensitivity to Compound 1.

[00156] Further studies showed that colorectal cancer cells lines with MSI-H status have significantly higher sensitivity to Compound 1 than those colorectal cancer cells lines that are

microsatellite stable (MSS). A panel of 13 colorectal cancer PDX models and 6 colorectal cancer organoid PDX models were tested for their sensitivity to Compound 1 in culture-based viability inhibition assays. The MSI-H status of the studied colorectal cancer cell lines and their respective mutations of the MMR path genes are shown in Fig. 19.

[00157] As summarized in Table 11 below, it was found that 100% of the PDX models characterized as MSI (5 out of 5) were sensitive (IC_{50} < 1 μM) to Compound 1 treatment and the IC_{50}'s ranged from 0.001 to 0.014 μM. However, only 50% of the PDX models characterized as MSS (4 out of 8) were sensitive to Compound 1 treatment and the IC_{50}'s ranged from 0.003 to 0.270 μM. Similarly, 100% of the organoid models characterized as MSI (3 out of 3) were sensitive to Compound 1 treatment (IC_{50} ranged from 0.022 to 0.149 μM), while none of the organoid models characterized as MSS (0 out of 3) was sensitive to Compound 1 treatment (IC_{50} values were greater than 10 μM).

Table 11

PDX Model Phenotype	Sensitive to Compound 1	IC_{50} (μM)[a]
MSI	5/5 (100%)	0.001 – 0.014
MSI (Organoid)	3/3 (100%)	0.022 – 0.149
MSS	4/8 (50%)	0.003 – 0.270
MSS (Organoid)	0/3 (0%)	> 10

Note: MSI – Microsatellite instability; MSS – Microsatellite stable

[a] IC_{50} shown only for those displaying sensitivity to Compound 1.

Example 6: Identification of biomarkers associated with Compound 1 treatment

[00158] RNA-seq and ChIP-seq analyses were employed to identify potential clinical pharmacodynamic (PD) biomarkers as molecular indicators for monitoring the effects of treatment with Compound 1.

[00159] In the RNA-seq analysis performed, 326 genes that were up- or downregulated ≥ 2-fold by Compound 1 treatment were initially identified. The tested dose of Compound 1 was 37.3 mg/kg, which was the highest dose tested in diffuse large B-Cell Lymphoma xenografts models. In the ChIP-seq analysis, 65 genes were identified to show direct KDM4C promoter occupancy. Fig. 20 shows that genes PNUTS (PPP1R10), ANK1, IBA57, SOWAHD, MF12-AS1, and CECR1 exhibited dose-dependent changes following treatment with Compound 1 *in vivo* at 2.5 mg/kg, 5 mg/kg, 7.5 mg/kg, 15 mg/kg, and 50 mg/kg. Fig. 20 also shows that

PNUTS, ANK1, and IBA57 are downregulated during Compound 1 treatment while SOWAHD, MF12-AS1, and CECR1 are upregulated.

[00160] Compound 1 treatment for 24 hours demonstrated potent inhibition of PNUTS gene expression. In a study of the inhibitory effects of Compound 1 on the gene expression of PNUTS, MDA-MB-231 cells were treated for 24 hours and expression levels of PNUTS mRNA in response to Compound 1 treatment were measured by qPCR. The inhibition of PNUTS mRNA was quantified by calculating mean percent change in PNUTS gene expression following the Compound 1 treatment relative to the DMSO-treated control and IC_{50} value was calculated as concentration where inhibition was half-maximal. The IC_{50} value was 0.007 μM.

[00161] Moreover, ChIP-seq analysis in MDA-MB-231 cells shows KDM4 occupancy at the PNUTS (PPP1R10) promoter as marked by H3K4me3 (Fig. 21)

Example 7: Single Cell Gene Expression Analysis of Immature and Mature Cell Markers

[00162] Vehicle control and Compound 1-treated samples were characterized by single cell transcriptional profiling with a gene-set designed to discriminate immature colon progenitor cells and more mature cell-types (Dalerba, 2011). Compound 1-treated tumors demonstrated a shift in cellularity upon KDM4C inhibition (Figure 22, panel A). Using hierarchical clustering analysis, three clusters based on expression of a subset of immature and mature cell markers were identified. Progenitor-like cell clusters were identified by the presence of high expression of immature cell markers (Lgr5, Notch1 and Ezh2) and low expression for mature cell markers (Krt20, Ceacam1, and Spink4). Intermediate progenitor-like cells were identified by the presence of high expression of Lgr5 but low expression of other immature cell markers such as Notch1, Ezh2. This cluster also expressed several markers of mature cells at higher levels than the progenitor-like cells but expressed cell cycle markers at lower levels suggesting that these cells were less proliferative than the progenitor-like cells (data not shown). Finally, a cluster of differentiated-like cells expressing low levels of immature cells markers (Lgr5, Notch1, Ezh2) and high levels of mature cells markers (Krt20/Ceacam1 and Tff3/ Dll1/Spink4) were identified. Compound 1-treated tumors had a significant reduction in the proportion of progenitor-like cells ($p = 0.01$) and an increase in number of both intermediate-like and differentiated-like cells although the increase was not significant ($p = 0.07$; Figure 22, panel B).

Example 8: Effect of Compound 1 on Hematopoietic Progenitor Cells

[00163] Compound 1 was evaluated for its effects on proliferation of erythroid and myeloid hematopoietic progenitor cells derived from human bone marrow at STEMCELL Technologies Inc. (Vancouver, BC, Canada).

[00164] Cells were thawed rapidly at 37 °C into DNAse I and then diluted in 10 mL of Iscove's Modified Dulbecco's medium containing 2% fetal bovine serum (IMDM + 2% FBS) and washed by centrifugation (1200 rpm for 10 minutes at room temperature). The supernatant was discarded and the cell pellet resuspended in a known volume of IMDM + 2% FBS. A nucleated cell count (3% glacial acetic acid) and viability assessment (trypan blue exclusive test) were performed.

[00165] Compound 1 and positive control (panobinostat) were diluted in DMSO and tested at concentrations ranging from 0.002 μM to 5 μM in final media. These dilutions provided the appropriate final test concentrations required for the test and control articles (5, 1.67, 0.56, 0.19, 0.06, 0.02, 0.07, and 0.002 μM).

[00166] Test and control articles were incubated with human bone marrow hematopoietic progenitor cells. After 14 days, cell colonies were assessed.

[00167] To calculate the concentration of 50% and 90% inhibition of colony growth (IC_{50} and IC_{90}) for each test article, a dose response curve was generated plotting the log of the test article concentration versus the percentage of control colony growth using GraphPad Prism. To generate a curve fitting these data points, a log (inhibitor) vs. response - variable slope (four parameters) equation [Y=Bottom + (Top-Bottom)/(1+10^((LogIC_{50}-X)*HillSlope))] was used. The IC_{50} and IC_{90} values for both test and control articles were derived from an interpolation of the curve fit. In cases where the $IC_{50/90}$ value reported by GraphPad Prism is a value beyond the range of concentrations that were actually tested, if this value is more than 4 times the highest concentration tested, or where GraphPad Prism cannot calculate an $IC_{50/90}$ value, notations of > 5 μM (for test article compound 1) are used.

[00168] Compound 1 had no significant effect compared to 0.1% DMSO on the colony formation of progenitor cells from normal human bone marrow ($p > 0.01$). As shown below in Table 12, IC_{50} values for inhibition of erythroid and myeloid colony formation were greater than 5 μM. Statistical analysis photographs taken of representative erythroid and myeloid derived colonies found that their morphology (size and density) was slightly compromised at compound 1 concentrations ≥ 1.67 μM. Panobinostat, the positive control, inhibited erythroid and myeloid colony formation with IC_{50} values less than 0.01 μM.

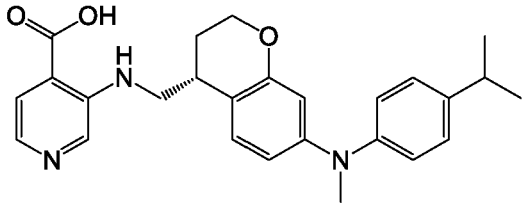
Table 12

Test Material	Erythroid		Myeloid	
	IC_{50} (μg/mL)	**IC_{90} (μg/mL)**	**IC_{50} (μg/mL)**	**IC_{90} (μg/mL)**
Compound 1	> 5	> 5	> 5	> 5
Panobinostat	0.0064	0.0075	0.0074	0.0081

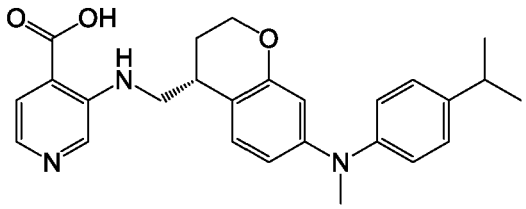
CLAIMS

WHAT IS CLAIMED IS:

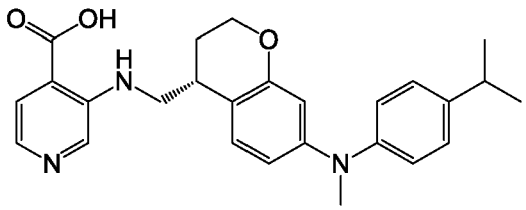
1. A method of treating a cancer in a cancer patient in need thereof, comprising administering to the patient a therapeutically effective amount of a compound, or a pharmaceutically acceptable salt or a solvate thereof, wherein the compound having the structure:



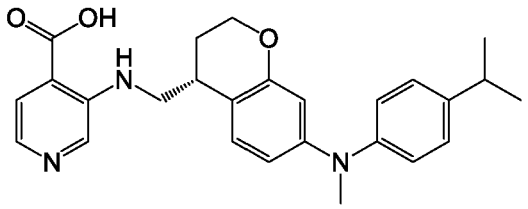
2. A method of reducing tumorigenic cell population in cancer a patient in need thereof, comprising administering to the patient therapeutically effective amount of a compound, or a pharmaceutically acceptable salt or a solvate thereof, wherein the compound having the structure:



3. A method of reducing tumor initiating cell frequency in a cancer patient in need thereof, comprising administering to the patient therapeutically effective amount of a compound, or a pharmaceutically acceptable salt or a solvate thereof, wherein the compound having the structure:



4. A method of inhibiting cancer stem cells in a cancer patient in need thereof, comprising administering to the patient therapeutically effective amount of a compound, or a pharmaceutically acceptable salt or a solvate thereof, wherein the compound having the structure:



5. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with a cancer selected from the group consisting of colorectal cancer, esophageal cancer, triple negative breast cancer, gastric cancer, lymphoma, gastric adenocarcinoma, diffuse large B-cell non-Hodgkin's lymphoma, acute T-cell leukemia, esophageal squamous cell carcinoma, multiple myeloma, acute myeloid leukemia, colorectal adenocarcinoma, colorectal carcinoma, pancreatic cancer, pancreatic carcinoma, breast carcinoma, and T-cell acute lymphoblastic leukemia.
6. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with colorectal cancer.
7. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with esophageal cancer.
8. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with triple negative breast cancer.
9. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with gastric cancer.
10. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with lymphoma.
11. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with gastric adenocarcinoma.
12. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with diffuse large B-cell non-Hodgkin's lymphoma.
13. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with acute T-cell leukemia.
14. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with esophageal squamous cell carcinoma.
15. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with multiple myeloma.
16. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with acute myeloid leukemia.

17. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with colorectal adenocarcinoma.
18. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with colorectal carcinoma.
19. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with pancreatic cancer.
20. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with pancreatic carcinoma.
21. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with breast carcinoma.
22. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with T-cell acute lymphoblastic leukemia.
23. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with a cancer selected from lung cancer, small cell lung cancer, non-small cell lung cancer, large cell lung cancer, lung adenocarcinoma, lung squamous cell carcinoma, lung small cell carcinoma, lung large cell carcinoma, or bronchioloalveolar adenocarcinoma.
24. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with a cancer selected from acute lymphoblastic B-cell leukemia, mantle cell lymphoma, plasma cell myeloma, diffuse large B-cell lymphoma, B-cell lymphoma, Burkitt lymphoma, blast phase chronic myeloid leukemia.
25. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with a cancer selected from intestinal cancer, intestinal adenocarcinoma, squamous cell carcinoma of the upper digestive tract.
26. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with a cancer selected from stomach cancer, stomach signet ring adenocarcinoma, adenocarcinoma of the stomach, or adenosquamous carcinoma.
27. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with a cancer selected from ovarian cancer, ovarian endometrioid carcinoma, ovarian clear cell carcinoma, ovarian adenocarcinoma, endometrial cancer, endometrial adenocarcinoma, prostate cancer, or prostate adenocarcinoma.
28. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with skin cancer.
29. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with a cancer selected from thyroid cancer, or thyroid follicular carcinoma.

30. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with a cancer selected from breast cancer, or breast ductal carcinoma.
31. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with a cancer selected from liver cancer, or hepatocellular carcinoma.
32. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with a cancer selected from a CNS cancer, astrocytoma grade IV, or gliosarcoma.
33. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with bone cancer.
34. The method of any one of claims 1-4, wherein the cancer patient has been diagnosed with a cancer selected from kidney cancer, clear cell renal cell carcinoma, renal cell carcinoma, urinary tract cancer, or urinary tract transitional cell carcinoma.
35. The method of any one of claims 1-34, wherein the cancer is relapsed after prior therapy, refractory to prior therapy, or acquired resistance to prior therapy.
36. The method of any one of claims 2, and 5-35, wherein the tumorigenic cell population is reduced from about 1.5-fold to about 100-fold.
37. The method of claim 36, wherein the tumorigenic cell population is reduced about 1.5-fold, about 2.0-fold, about 2.5-fold, about 3.0-fold, about 3.5-fold, about 4.0-fold, about 4.5-fold, about 5.0-fold, about 6.0-fold, about 7.0-fold, about 8.0-fold, about 9.0-fold, about 10-fold, about 15-fold, about 20-fold, about 25-fold, about 30-fold, about 35-fold, about 40-fold, about 45-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, or about 100-fold.
38. The method of claim 36, wherein the tumorigenic cell population is reduced about 1.5-fold, about 2.0-fold, about 2.5-fold, about 3.0-fold, about 3.5-fold, about 4.0-fold, about 4.5-fold, or about 5.0-fold.
39. The method of any one of claims 3, and 5-35, wherein the tumor initiating cell frequency is reduced from about 1.5-fold to about 100-fold.
40. The method of claim 39, wherein the tumor initiating cell frequency is reduced about 1.5-fold, about 2.0-fold, about 2.5-fold, about 3.0-fold, about 3.5-fold, about 4.0-fold, about 4.5-fold, about 5.0-fold, about 6.0-fold, about 7.0-fold, about 8.0-fold, about 9.0-fold, about 10-fold, about 15-fold, about 20-fold, about 25-fold, about 30-fold, about 35-fold, about 40-fold, about 45-fold, about 50-fold, about 60-fold, about 70-fold, about 80-fold, about 90-fold, or about 100-fold.
41. The method of claim 39, wherein the tumor initiating cell frequency is reduced about 1.5-fold, about 2.0-fold, about 2.5-fold, about 3.0-fold, about 3.5-fold, about 4.0-fold,

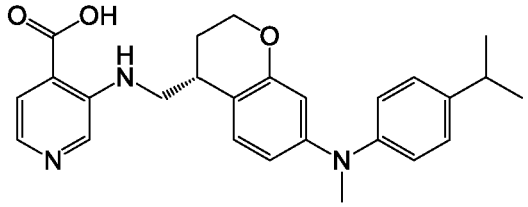
about 4.5-fold, about 5.0-fold, about 6.0-fold, about 7.0-fold, about 8.0-fold, about 9.0-fold, or about 10-fold.

42. The method of any one of claims 3, and 5-35, wherein the tumor initiating cell frequency is reduced to a frequency of about 1-in-100 cells to about 1-in-10,000 cells.

43. The method of claim 42, wherein the tumor initiating cell frequency is reduced to a frequency of about 1-in-100 cells, about 1-in-200 cells, about 1-in-300 cells, about 1-in-400 cells, about 1-in-500 cells, about 1-in-600 cells, about 1-in-700 cells, about 1-in-800 cells, about 1-in-900 cells, about 1-in-1,000 cells, about 1-in-1,200 cells, about 1-in-1,400 cells, about 1-in-1,600 cells, about 1-in-1,800 cells, about 1-in-2,000 cells, about 1-in-3,000 cells, about 1-in-4,000 cells, about 1-in-5,000 cells, about 1-in-6,000 cells, about 1-in-7,000 cells, about 1-in-8,000 cells, about 1-in-9,000 cells, or to about 1-in-10,000 cells.

44. The method of claim 42, wherein the tumor initiating cell frequency is reduced to a frequency of about 1-in-200 cells, about 1-in-300 cells, about 1-in-400 cells, about 1-in-500 cells, about 1-in-600 cells, about 1-in-700 cells, about 1-in-800 cells, about 1-in-900 cells, about 1-in-1,000 cells, or about 1-in-1,200 cells.

45. A method of treating a cancer in a cancer patient in need thereof, comprising:
(a) determining high microsatellite instability (MSI-H) status of the cancer patient, and
(b) administering therapeutically effective amount of a compound or a pharmaceutically acceptable salt or a solvate thereof to the cancer patient if the cancer patient is determined to be a MSI-H cancer patient, wherein the compound having the structure:



46. A method of treating cancer, comprising:
(a) obtaining a sample from a patient having the cancer;
(b) determining the level of a biomarker in the sample;
(c) diagnosing the patient as being likely to be responsive to a treatment compound if the level of the biomarker in the sample is different from a reference level of the biomarker; and (d) administering a therapeutically effective amount of the treatment compound to the patient; and

wherein the treatment compound is Compound 1, or a pharmaceutically acceptable salt or a solvate thereof; and

the biomarker is PNUTS.

47. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with a cancer selected from the group consisting of colorectal cancer, esophageal cancer, triple negative breast cancer, gastric cancer, lymphoma, gastric adenocarcinoma, diffuse large B-cell non-Hodgkin's lymphoma, acute T-cell leukemia, esophageal squamous cell carcinoma, multiple myeloma, acute myeloid leukemia, colorectal adenocarcinoma, colorectal carcinoma, pancreatic cancer, pancreatic carcinoma, breast carcinoma, and T-cell acute lymphoblastic leukemia.
48. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with a cancer selected from lung cancer, small cell lung cancer, non-small cell lung cancer, large cell lung cancer, lung adenocarcinoma, lung squamous cell carcinoma, lung small cell carcinoma, lung large cell carcinoma, or bronchioloalveolar adenocarcinoma.
49. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with a cancer selected from acute lymphoblastic B-cell leukemia, mantle cell lymphoma, plasma cell myeloma, diffuse large B-cell lymphoma, B-cell lymphoma, Burkitt lymphoma, blast phase chronic myeloid leukemia.
50. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with a cancer selected from intestinal cancer, intestinal adenocarcinoma, squamous cell carcinoma of the upper digestive tract.
51. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with a cancer selected from stomach cancer, stomach signet ring adenocarcinoma, adenocarcinoma of the stomach, or adenosquamous carcinoma.
52. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with a cancer selected from ovarian cancer, ovarian endometrioid carcinoma, ovarian clear cell carcinoma, ovarian adenocarcinoma, endometrial cancer, endometrial adenocarcinoma, prostate cancer, or prostate adenocarcinoma.
53. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with skin cancer.
54. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with a cancer selected from thyroid cancer, or thyroid follicular carcinoma.
55. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with a cancer selected from breast cancer, or breast ductal carcinoma.

56. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with a cancer selected from liver cancer, or hepatocellular carcinoma.
57. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with a cancer selected from a CNS cancer, astrocytoma grade IV, or gliosarcoma.
58. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with bone cancer.
59. The method of claim 33 or 34, wherein the cancer patient has been diagnosed with a cancer selected from kidney cancer, clear cell renal cell carcinoma, renal cell carcinoma, urinary tract cancer, or urinary tract transitional cell carcinoma.
60. The method of claim 33 or 34, wherein the cancer is relapsed after prior therapy, refractory to prior therapy, or acquired resistance to prior therapy.

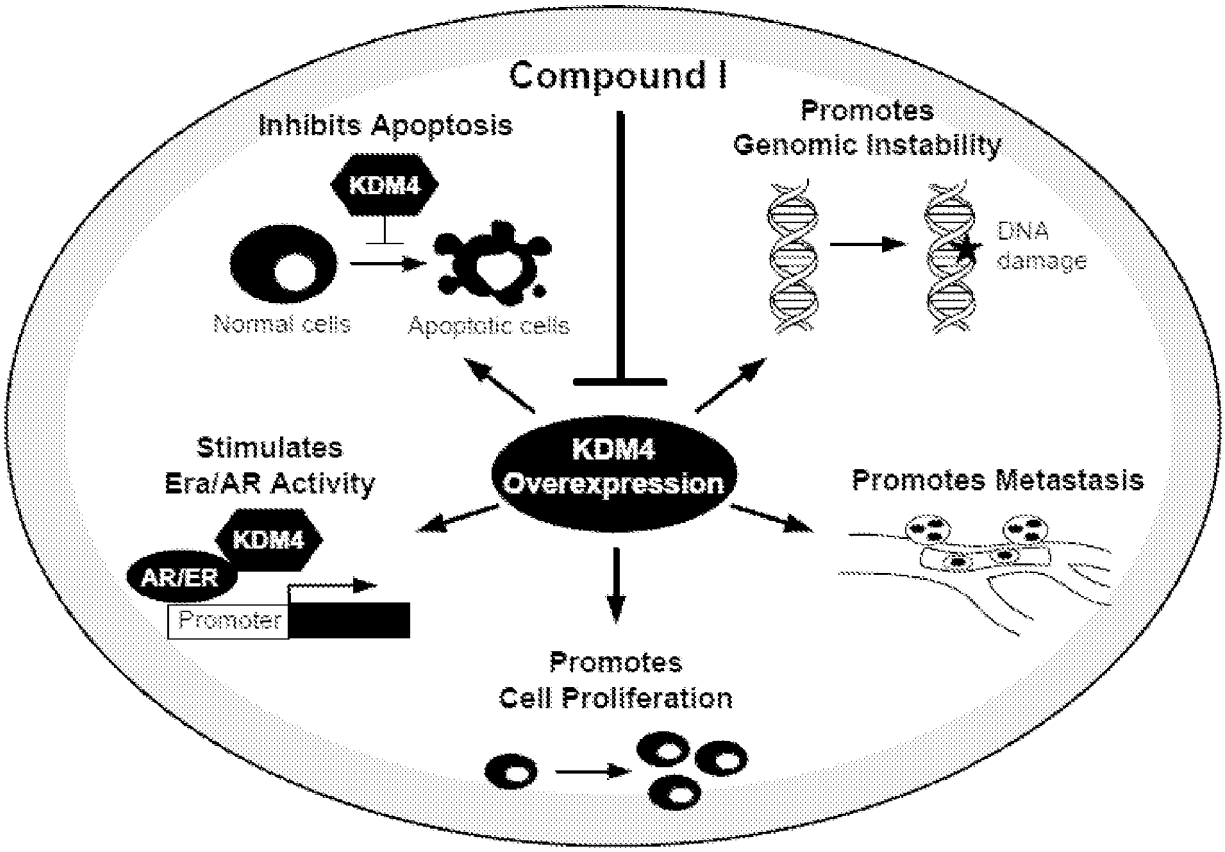

Compound I
Inhibits Apoptosis
KDM4
Normal cells
Apoptotic cells
Promotes
Genomic Instability
DNA
damage
Stimulates
Era/AR Activity
KDM4
AR/ER
Promoter
KDM4
Overexpression
Promotes Metastasis
Promotes
Cell Proliferation

FIG. 1

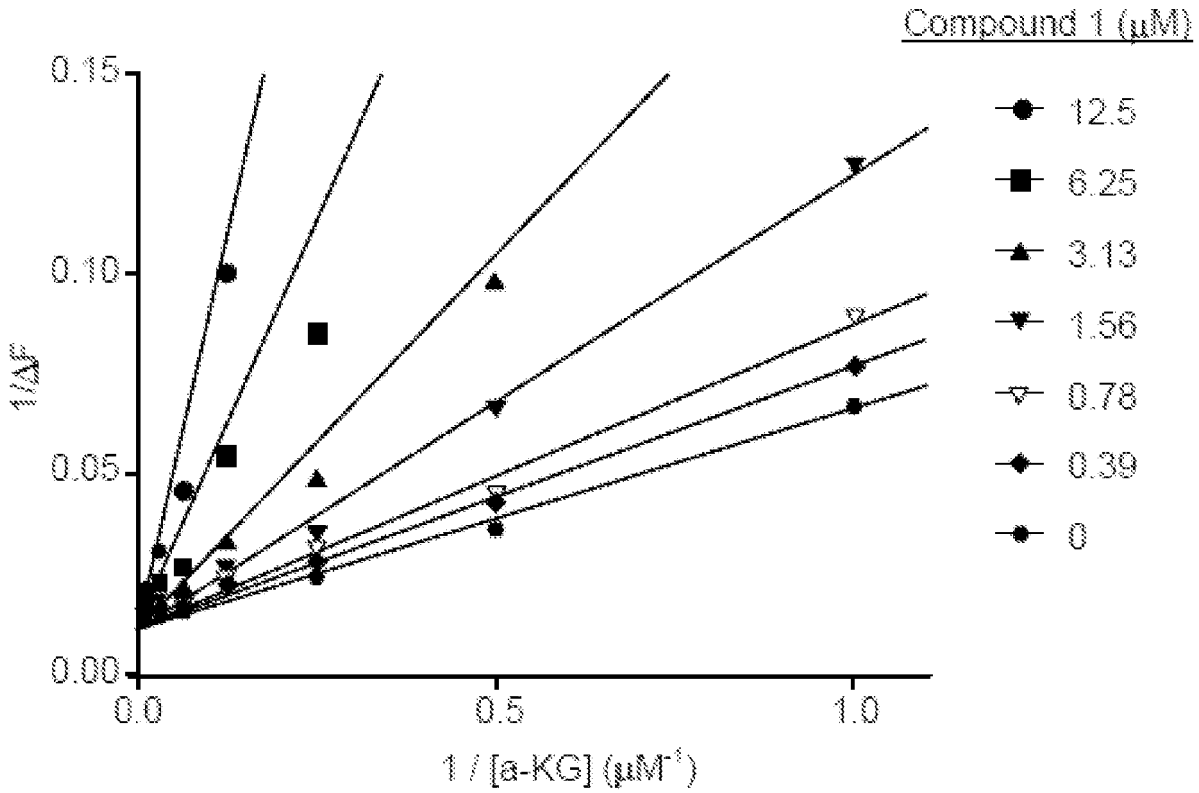

Compound 1 (μM)
12.5
6.25
3.13
1.56
0.78
0.39
0
0.15
0.10
0.05
0.00
1/ΔF
0.0
0.5
1.0
1 / [α-KG] (μM-1)

FIG. 2

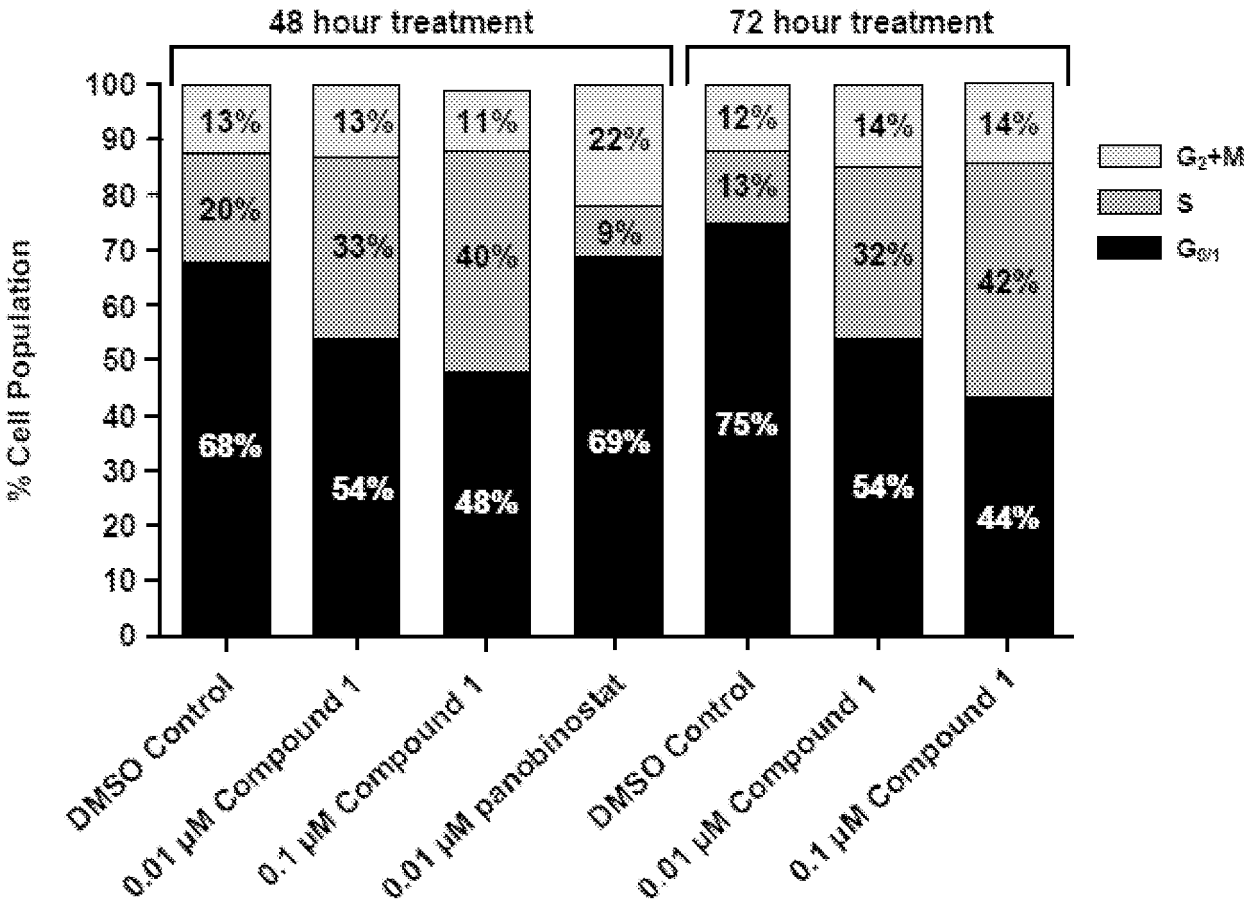

48 hour treatment
72 hour treatment
% Cell Population
100
90
80
70
60
50
40
30
20
10
0
13%
20%
68%
13%
33%
54%
11%
40%
48%
22%
9%
69%
12%
13%
75%
14%
32%
54%
14%
42%
44%
G2+M
S
G0/1
DMSO Control
0.01 µM Compound 1
0.1 µM Compound 1
0.01 µM panobinostat
DMSO Control
0.01 µM Compound 1
0.1 µM Compound 1

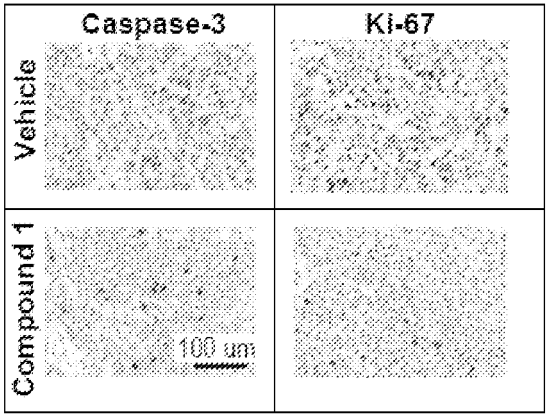
FIG. 3

Half-Maximal Effective Concentration for Apoptosis in Human Cancer Cell Lines

Cell Line	Description	IC50 (μM)
KYSE-150	Human esophageal squamous cell carcinoma	0.033
MDA-MB-231	Human triple negative breast cancer	0.132
HT-29	Human colorectal adenocarcinoma	0.092
HCC1937	Human triple negative breast cancer	>100

Apoptosis in human cancer cell lines were investigated in the presence or absence of Compound 1 using Caspase-Glo® 3/7 assay.

Representative IHC for Cleaved Caspase-e and Ki-67 in KYSE-150 Xenograft model.



Bottom Panels: Compound 1 treatment induced a 2-fold increase in apoptotic cells (p = 0.04) and a 2.4-fold decrease in proliferating cells (p = 0.006) compared to control group.

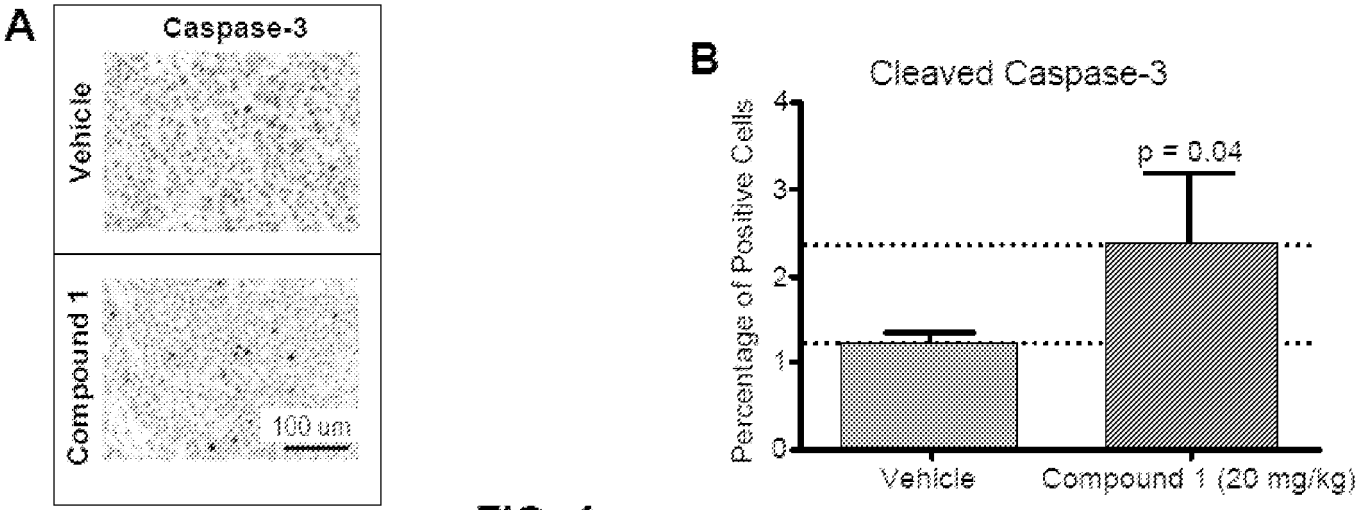


FIG. 4

Cell Line	Human Cancer Type	IC50 (μM)
Jurkat	Acute T cell Leukemia	0.0027
MDA-MB-231	Triple Negative Breast Cancer	0.0035
SNU-1	Gastric Cancer	0.0040
KYSE-150	Esophageal Squamous Cell Carcinoma	0.0053
MM.1s	Multiple Myeloma	0.0090
HL-60	Acute Myeloid Leukemia	0.0130
HT-29	Colorectal Adenocarcinoma	0.0235
MCF-7	Breast Carcinoma	0.0370
Loucy	T-acute lymphoblastic leukemia	< 0.01
TMD8	Diffuse Large B Cell Lymphoma (Type ABC)	0.0100
OCI-LY-19	Diffuse Large B Cell Lymphoma (Type GCB)	0.0100
U-2940	Diffuse Large B Cell Lymphoma (Type PMBL)	0.0200
IMR-90	(normal) fetal lung fibroblast	> 1.0

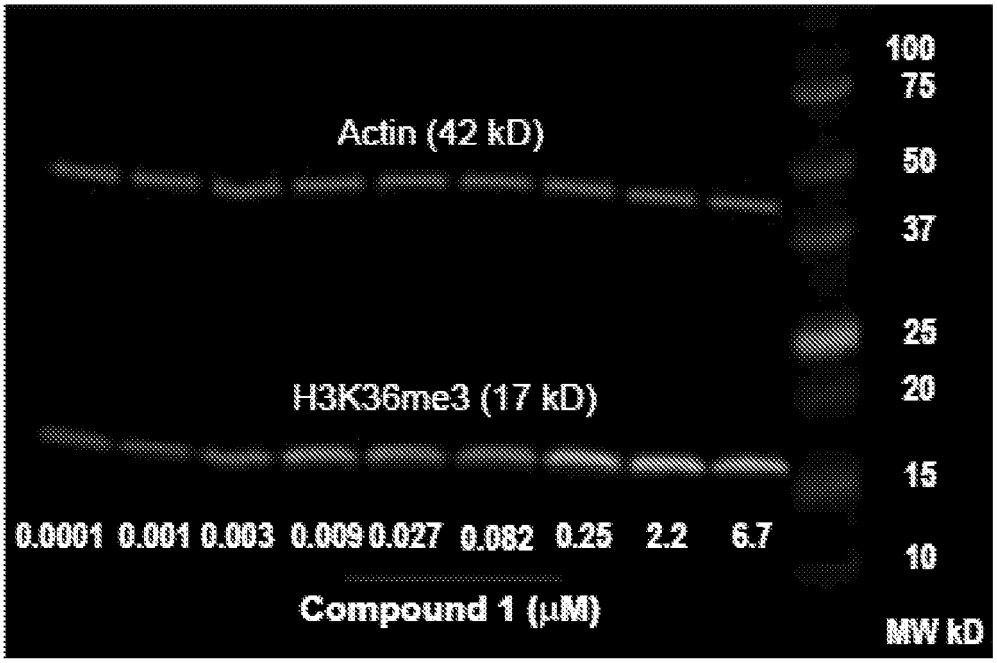
Highly selective potency profile (absolute IC_{50} values ranging from <500 pM to >10 μM). Active in both solid tumors and hematological cancers

Sensitivity of compound 1 in GI Cancers

Cancer Type	Sensitivity to compound 1	IC_{50} range (μM)
Cell lines		
Gastric	20/22 (91%)	0.004-0.146
Small/Large Intestine	23/28 (82%)	0.002-0.058
Pancreas	8/10 (80%)	0.004-0.116
Esophageal	15/19 (79%)	0.003-0.022
Liver	9/12 (75%)	0.011-0.129
PDX/Organoid Models		
Gastric (PDX)	4/5 (80%)	0.007-0.039
MSI CRC (PDX)	5/5 (100%)	0.001-0.014
MSI CRC (Organoid)	3/3 (100%)	0.022-0.149
MSS CRC (PDX)	4/8 (50%)	0.003-0.270
MSS CRC (Organoid)	0/3 (0%)	>10

FDX = Patient-derived Xenograft; CRC – Colorectal Cancer; MSI = Microsatellite Instability; MSS = Microsatellite Stable

FIG. 5


Actin (42 kD)
H3K36me3 (17 kD)
0.0001 0.001 0.003 0.009 0.027 0.082 0.25 2.2 6.7
Compound 1 (μM)
100
75
50
37
25
20
15
10
MW kD

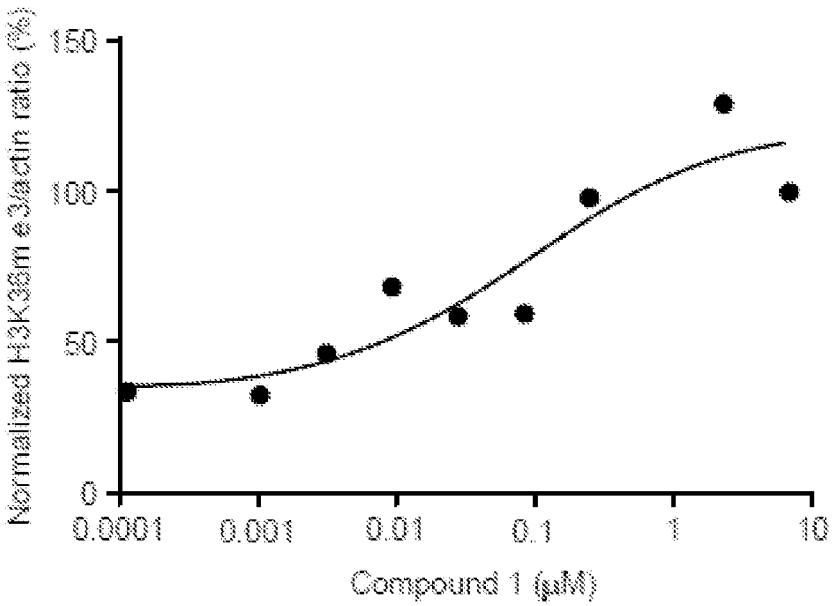
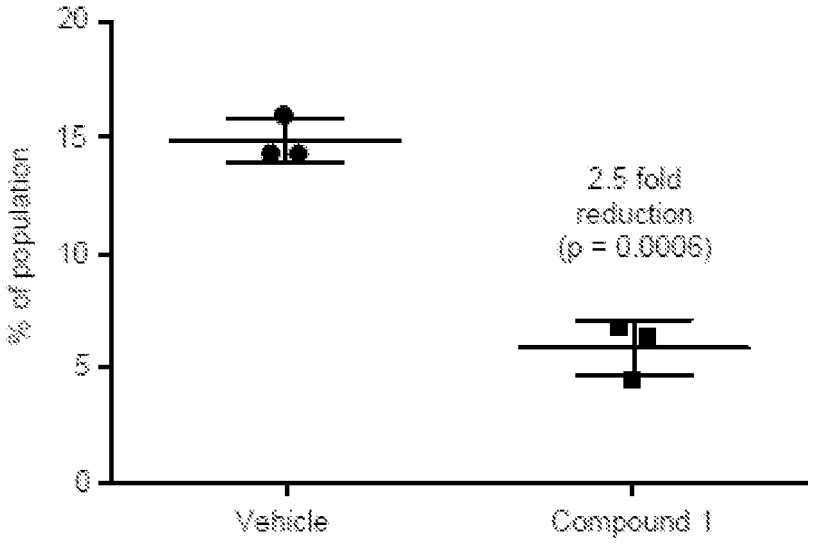

Normalized H3K36me3/actin ratio (%)
150
100
50
0
0.0001
0.001
0.01
0.1
1
10
Compound 1 (μM)

FIG. 6



Flow analysis of $CD44^{high}$ $EpCAM^{+}$ cells from SU60 Xenograft Tumors post Compound 1 treatment.

FIG. 7A

Compound 1 reduced Tumor Initiating Cell (TIC) frequency by 4.4 fold

LIMITING DILUTION ASSAY Treatment Group		1000 Cells	500 Cells	50 Cells	TIC Frequency
Vehicle	Tumor Take Rate	10/10 (100%)	8/10 (80%)	8/10 (80%)	1 in 144 cells
	Mean Tumor Vol	1588 mm^3	1290 mm^3	984 mm^3	
Compound 1 (40 mg/kg QD)	Tumor Take Rate	7/10 (70%)	5/10 (50%)	3/10 (30%)	1 in 643 cells
	Mean Tumor Vol	830 mm^3	589 mm^3	209 mm^3	

Tumorigenic potential of SU60 xenograft cells post Compound 1 treatment was evaluated by injecting in a limiting dilution manner into recipient NSG mice; number of cells required for tumor formation and tumor volumes were measured.

FIG. 7B

In Vivo Efficacy Seen in Multiple Cancer Models

Xenograft Model	Cancer Type	Dosing Schedule	Doses (mg/kg)	Efficacy (TGI%)	P-value (treated vs. control)
SU60 PDX	CRC	QD, QOD, 3on/4off	10, 14, 20, 23	29% - 71%	<0.0001 to 0.005
KYSE-150 CDX	ESCC	3on/4off	5, 15, 50	19% - 72%	<0.0001 to 0.054
	ESCC	QD	10, 15, 20	54% - 84%	<0.0001 to 0.015
COH70 PDX	TNBC	QD	12.5, 25, 40, 50	57% - 86%	<0.0001
GXA-3036 PDX	GC	3on/4off, 2on/5off	5, 15, 22.5, 50	41% - 69%	0.0007 to >0.05
OCI-LY19 CDX	DLBCL	QD 3on/4off, BID 3on/4off	5, 15, 25, 50	55% - 102%	<0.0001

CRC = colorectal cancer. ESCC = Esophageal squamous cell carcinoma, TNBC = Triple negative breast cancer; GC = Gastric adenocarcinoma. DLBCL = Diffuse large B-cell non-Hodgkin's lymphoma; CDX = cell-derived xenograft; PDX = patient-derived xenograft

FIG. 8

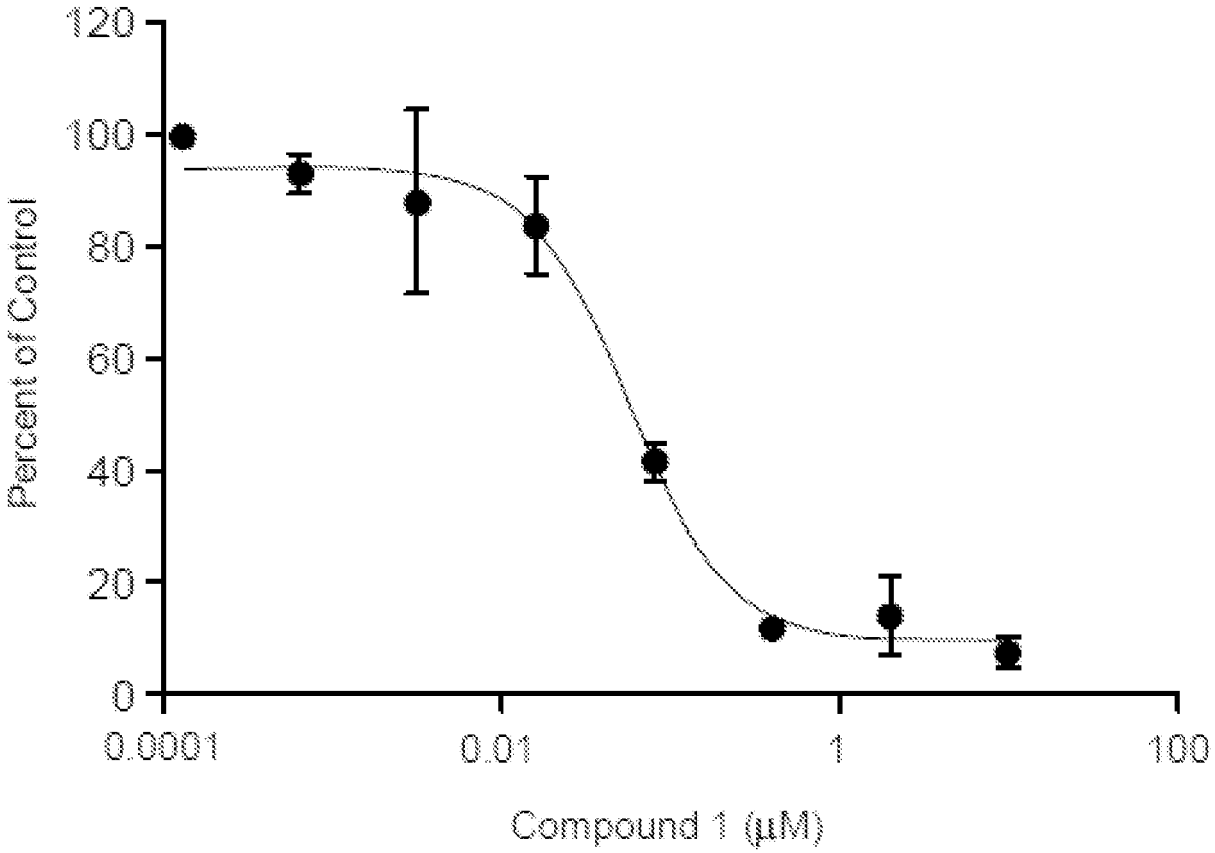

120
100
80
60
40
20
0
Percent of Control
0.0001
0.01
1
100
Compound 1 (μM)

FIG. 9

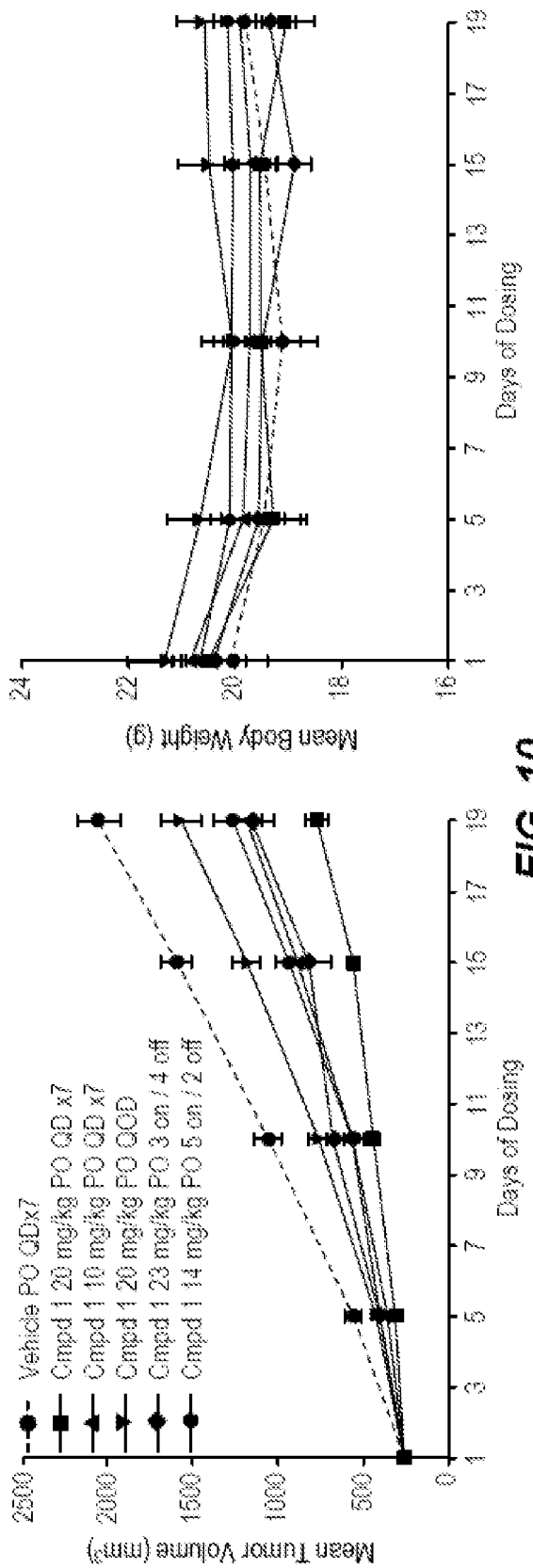

Vehicle PO QDx7
Cmpd 1 20 mg/kg PO QD x7
Cmpd 1 10 mg/kg PO QD x7
Cmpd 1 20 mg/kg PO QOD
Cmpd 1 23 mg/kg PO 3 on / 4 off
Cmpd 1 14 mg/kg PO 5 on / 2 off
Mean Tumor Volume (mm³)
Days of Dosing
Mean Body Weight (g)
Days of Dosing

FIG. 10

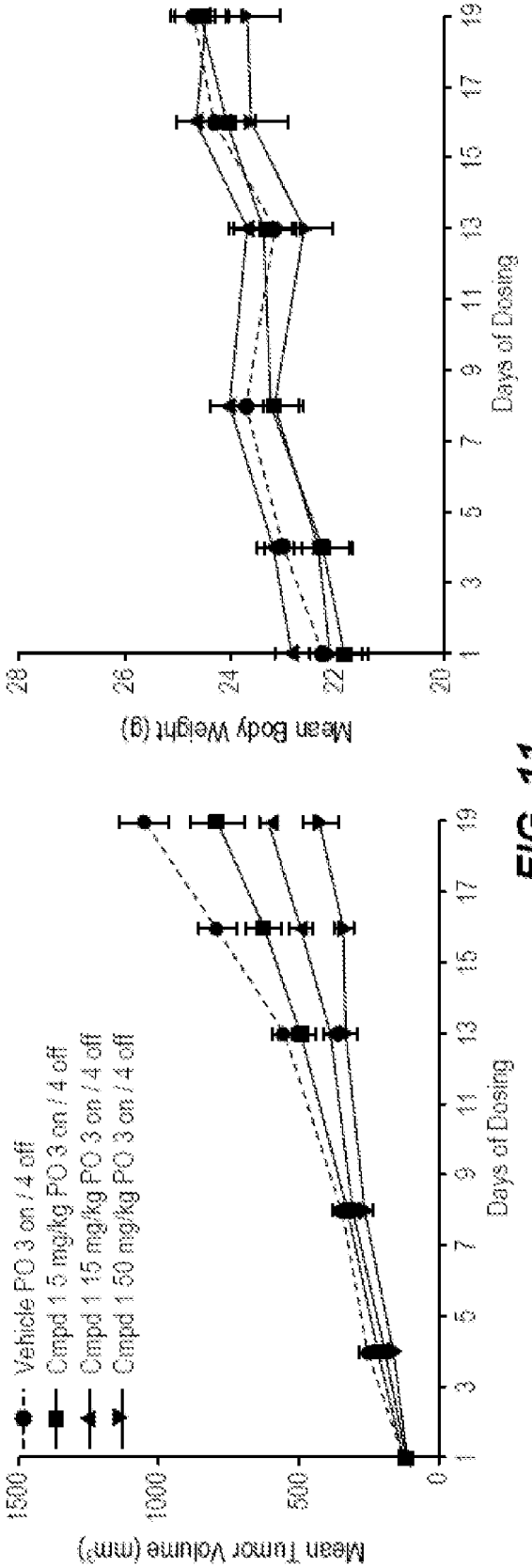

Vehicle PO 3 on / 4 off
Cmpd 1 5 mg/kg PO 3 on / 4 off
Cmpd 1 15 mg/kg PO 3 on / 4 off
Cmpd 1 50 mg/kg PO 3 on / 4 off
Mean Tumor Volume (mm³)
Days of Dosing
Mean Body Weight (g)
Days of Dosing

FIG. 11

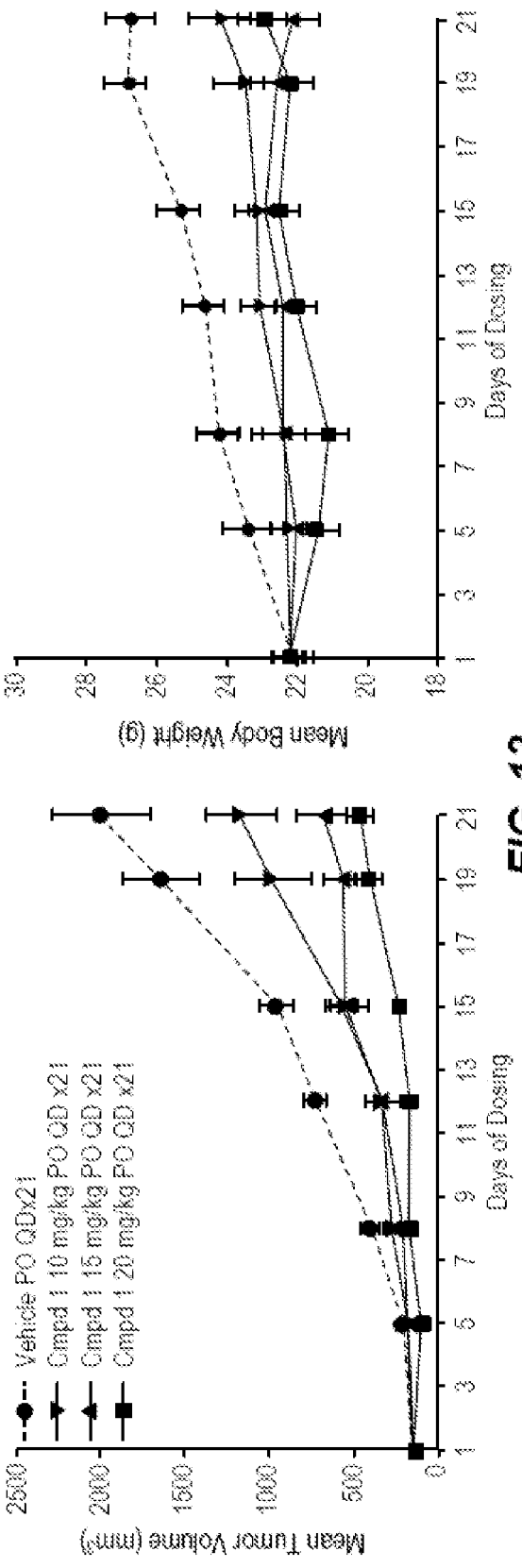

Mean Body Weight (g)
Days of Dosing
Mean Tumor Volume (mm³)
Days of Dosing
Vehicle PO QDx21
Cmpd 1 10 mg/kg PO QD x21
Cmpd 1 15 mg/kg PO QD x21
Cmpd 1 20 mg/kg PO QD x21

FIG. 12

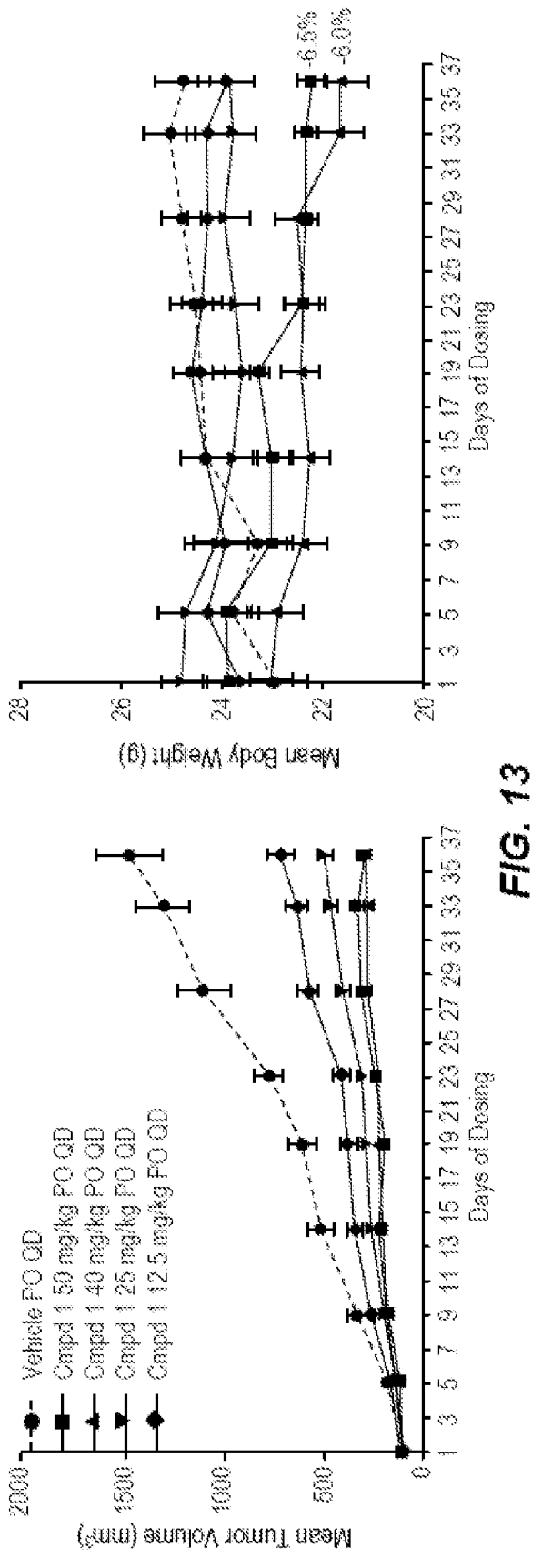

-6.5%
-6.0%
Mean Body Weight (g)
Days of Dosing
Mean Tumor Volume (mm³)
Days of Dosing
Vehicle PO QD
Cmpd 1 50 mg/kg PO QD
Cmpd 1 40 mg/kg PO QD
Cmpd 1 25 mg/kg PO QD
Cmpd 1 12.5 mg/kg PO QD

FIG. 13

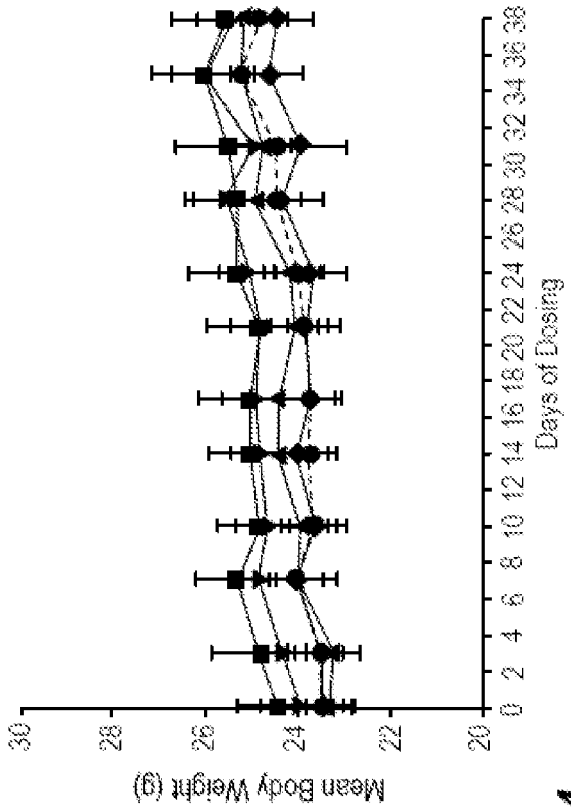

Mean Body Weight (g)
Days of Dosing
Mean Tumor Volume (mm³)
Days of Dosing
Vehicle
Cmpd 1 at 5 mg/kg 3 on / 4 off
Cmpd 1 at 15 mg/kg 3 on / 4 off
Cmpd 1 at 50 mg/kg 3 on / 4 off
Cmpd 1 at 22.5 mg/kg 2 on / 5 off

FIG. 14

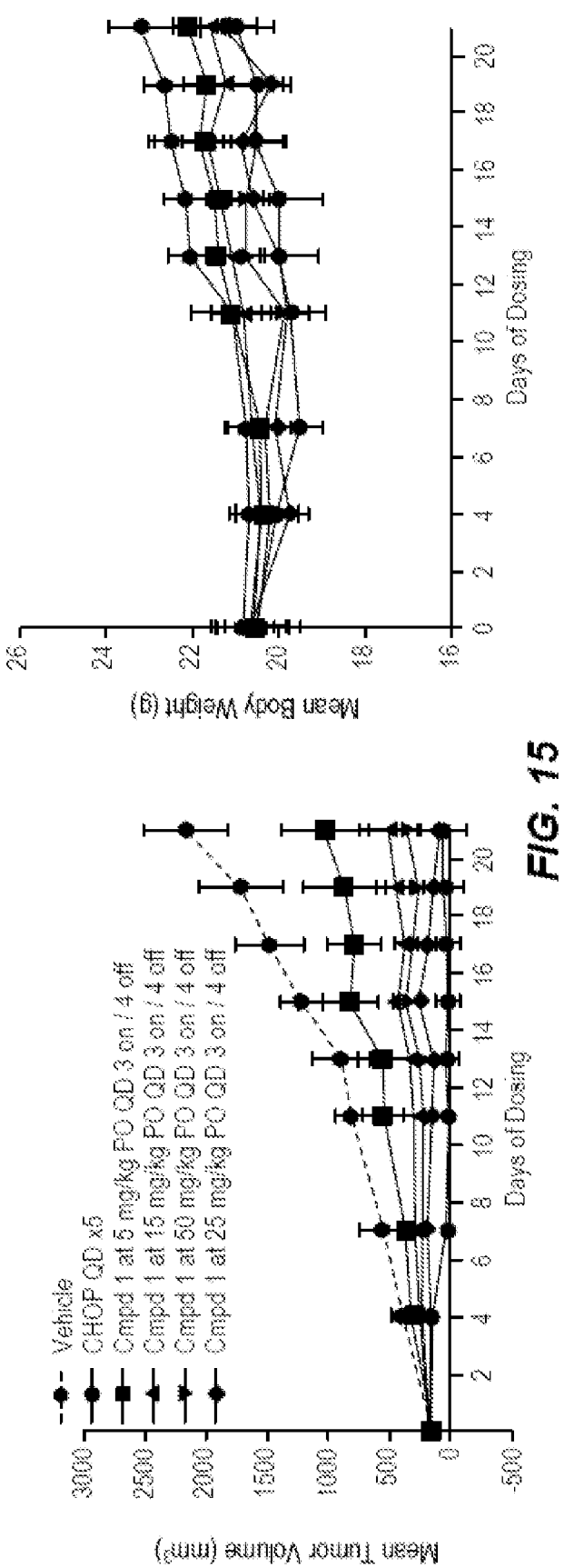

Mean Body Weight (g)
Days of Dosing
Mean Tumor Volume (mm³)
Days of Dosing
Vehicle
CHOP QD x5
Cmpd 1 at 5 mg/kg PO QD 3 on / 4 off
Cmpd 1 at 15 mg/kg PO QD 3 on / 4 off
Cmpd 1 at 50 mg/kg PO QD 3 on / 4 off
Cmpd 1 at 25 mg/kg PO QD 3 on / 4 off

FIG. 15

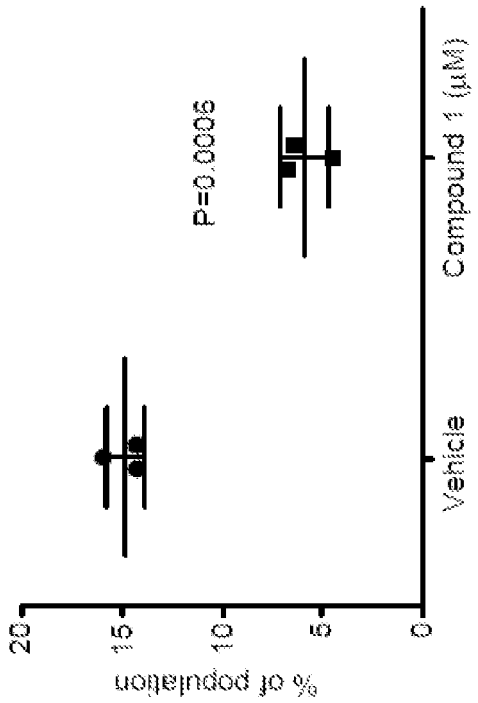

% of population
20
15
10
5
0
P=0.0006
Vehicle
Compound 1 (μM)

FIG. 16

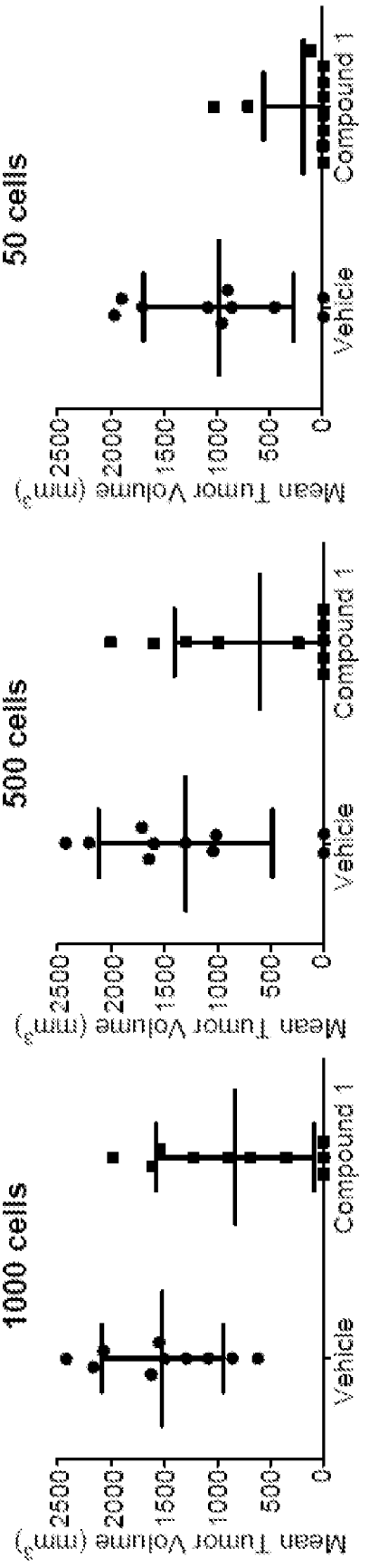

50 cells
500 cells
1000 cells
Mean Tumor Volume (mm³)
2500
2000
1500
1000
500
0
Vehicle
Compound 1

FIG. 17

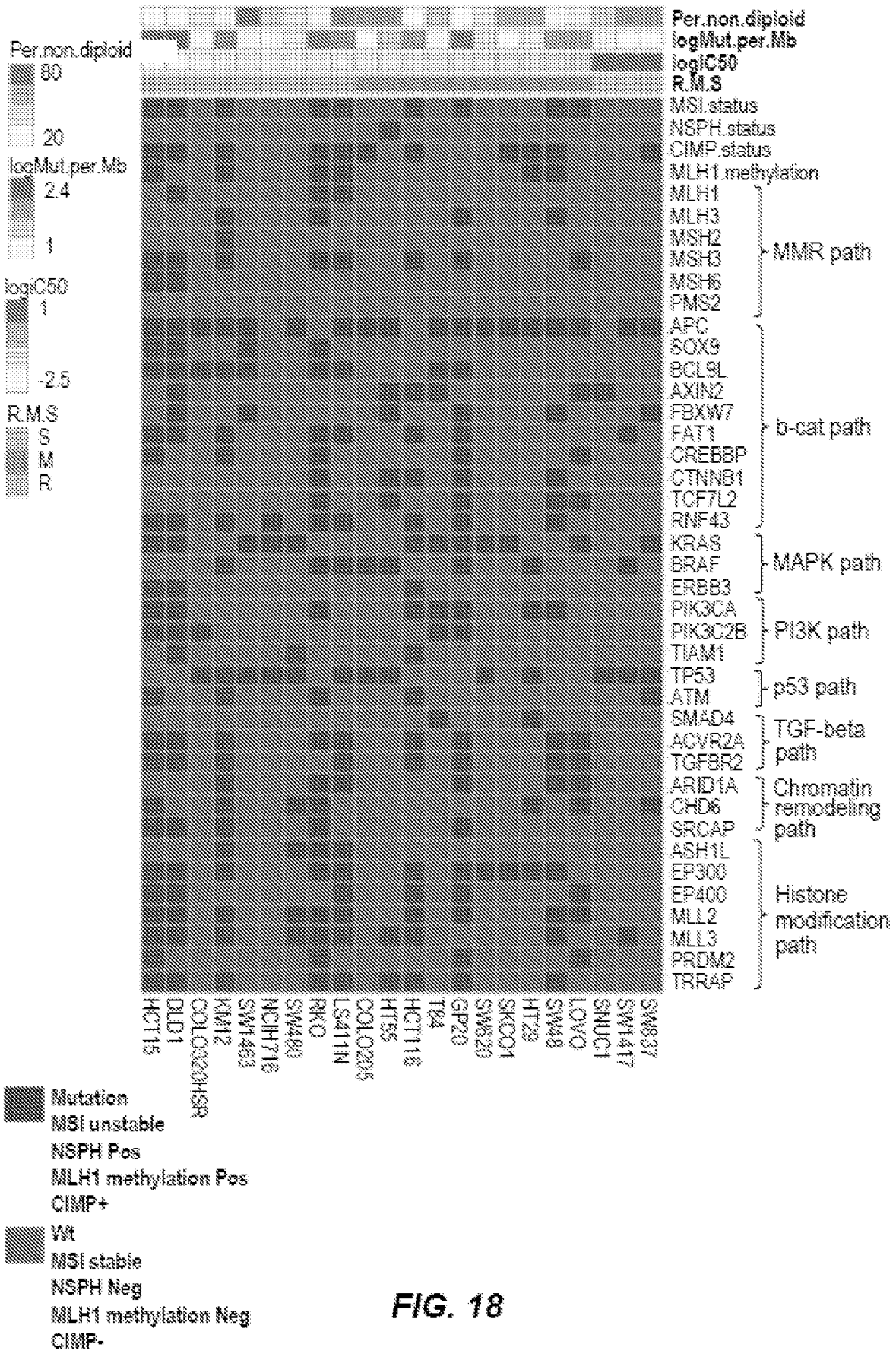

Per.non.diploid
80
20
logMut.per.Mb
2.4
1
logIC50
1
-2.5
R.M.S
Per.non.diploid
logMut.per.Mb
logIC50
R.M.S
MSI.status
NSPH.status
CIMP.status
MLH1.methylation
MLH1
MLH3
MSH2
MSH3
MSH6
PMS2
MMR path
APC
SOX9
BCL9L
AXIN2
FBXW7
FAT1
CREBBP
CTNNB1
TCF7L2
RNF43
b-cat path
KRAS
BRAF
ERBB3
MAPK path
PIK3CA
PIK3C2B
TIAM1
PI3K path
TP53
ATM
p53 path
SMAD4
ACVR2A
TGFBR2
TGF-beta path
ARID1A
CHD6
SRCAP
Chromatin remodeling path
ASH1L
EP300
EP400
MLL2
MLL3
PRDM2
TRRAP
Histone modification path
HCT15
DLD1
COLO320HSR
KM12
SW1463
NCIH716
SW480
RKO
LS411N
COLO205
HT55
HCT116
T84
GP20
SW620
SKCO1
HT29
SW48
LOVO
SNUC1
SW1417
SW837
Mutation
MSI unstable
NSPH Pos
MLH1 methylation Pos
CIMP+
Wt
MSI stable
NSPH Neg
MLH1 methylation Neg
CIMP-

FIG. 18

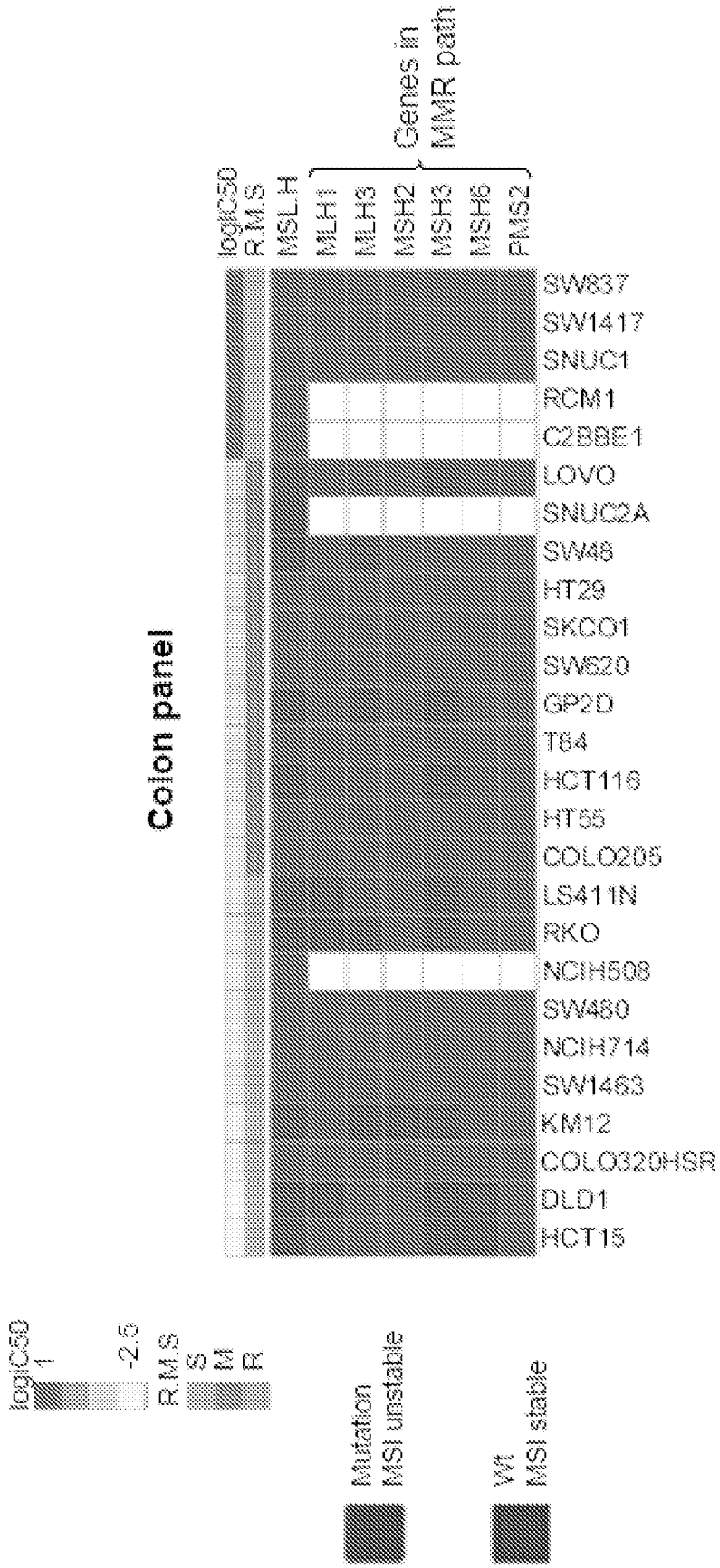

Colon panel
logIC50
R.M.S
MSI.H
MLH1
MLH3
MSH2
MSH3
MSH6
PMS2
Genes in MMR path
SW837
SW1417
SNUC1
RCM1
C2BBE1
LOVO
SNUC2A
SW48
HT29
SKCO1
SW620
GP2D
T84
HCT116
HT55
COLO205
LS411N
RKO
NCIH508
SW480
NCIH714
SW1463
KM12
COLO320HSR
DLD1
HCT15
logIC50
1
-2.5
R.M.S
S
M
R
Mutation
MSI unstable
Wt
MSI stable

FIG. 19

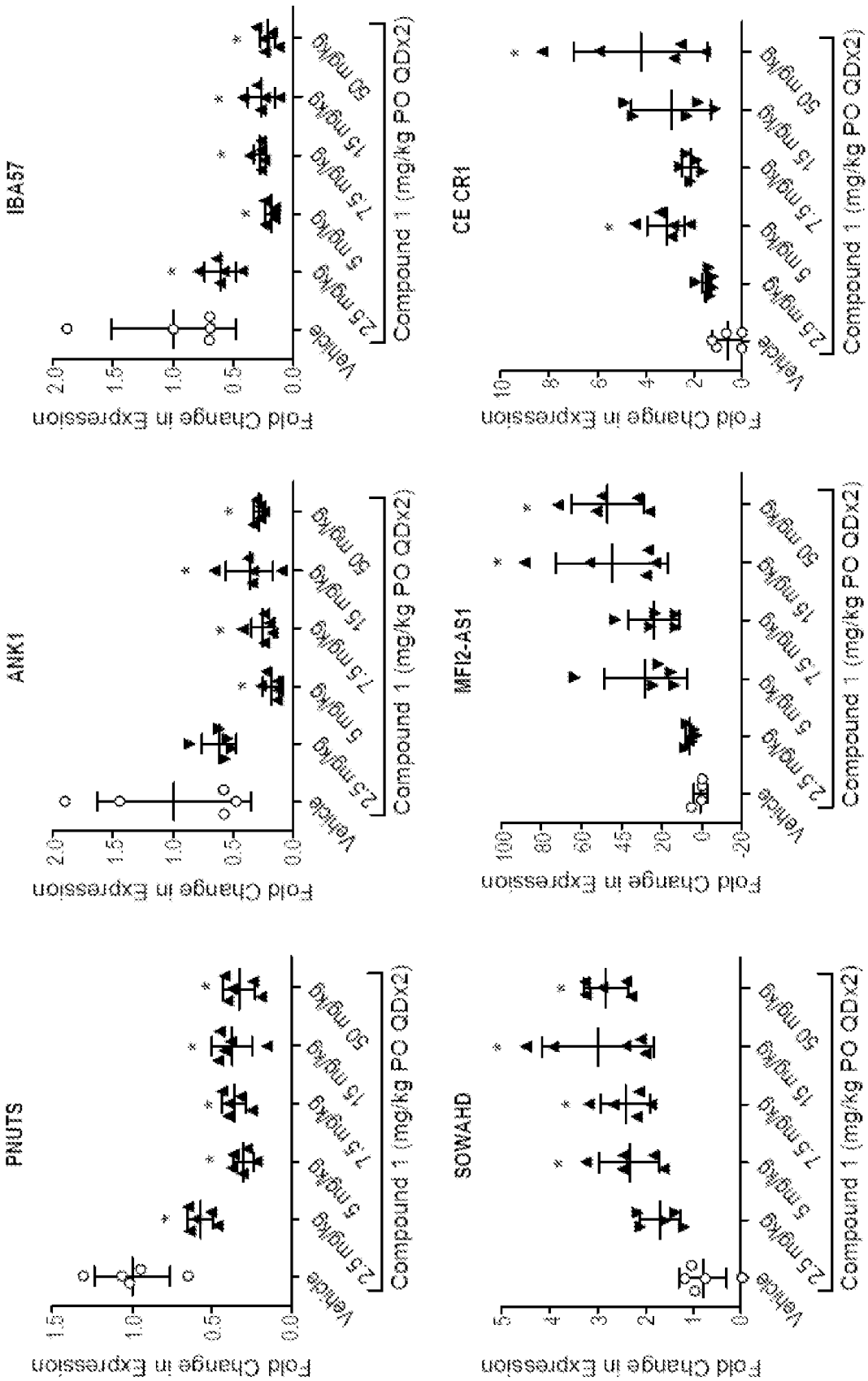
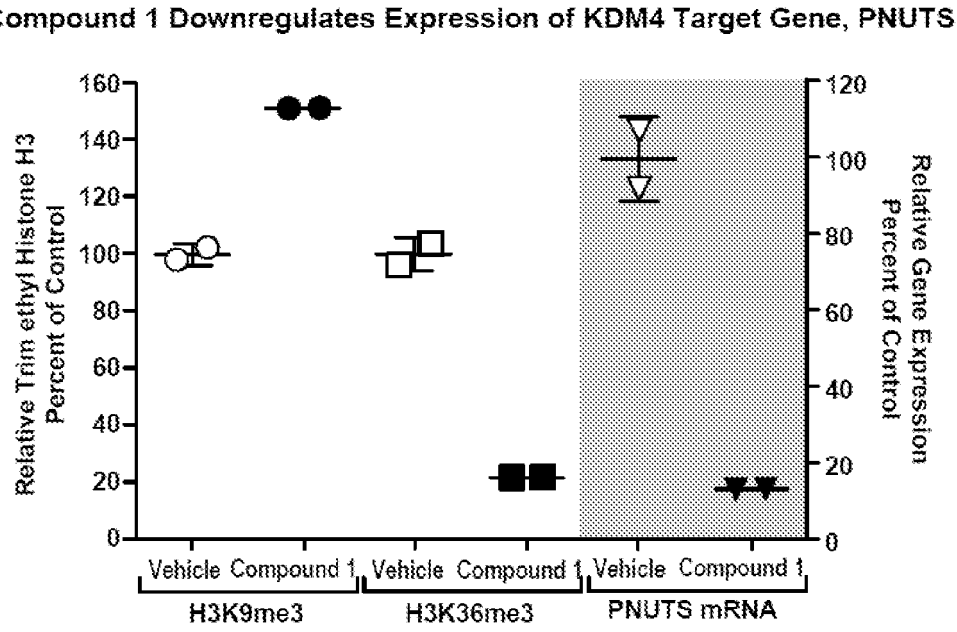

IBA57
AMK1
PNUTS
CE CR1
MFI2-AS1
SOWAHD
Fold Change in Expression
Vehicle
2.5 mg/kg
5 mg/kg
7.5 mg/kg
15 mg/kg
50 mg/kg
Compound 1 (mg/kg PO QDx2)

FIG. 20



KYSE-150 ESCC cells were simultaneously assayed for PNUTS gene expression by qPCR and histone methylation by ChIP-qPCR following treatment with Compound 1. Results indicate a 86% repression of PNUTS mRNA level, as well as 51% increase in H3K9me3, a mark of repressed transcription. A 78% decrease in H3K36me3 binding at the PNUTS gene also was observed.

FIG. 20
(Continued)

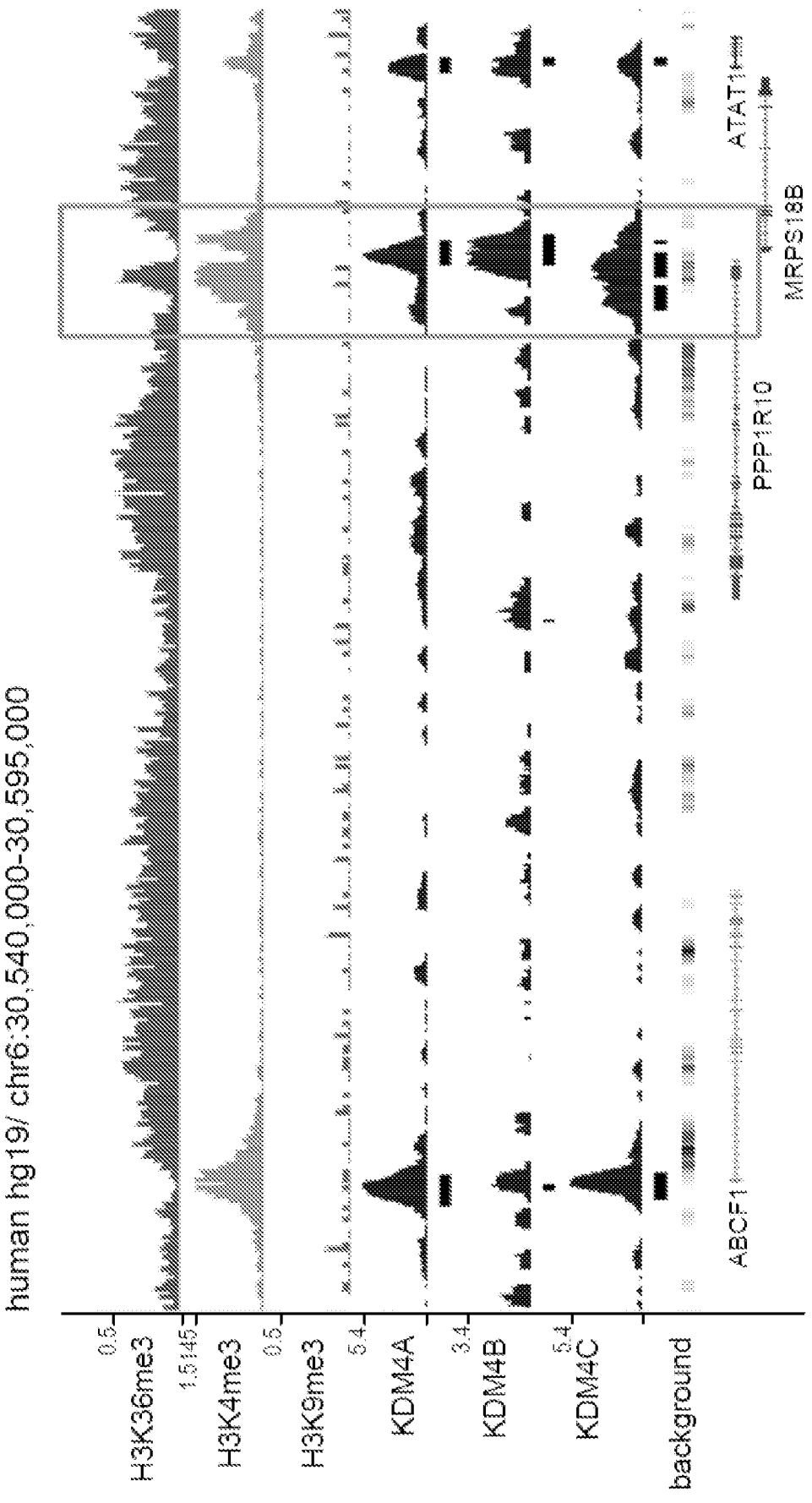

human hg19/ chr6:30,540,000-30,595,000
H3K36me3
H3K4me3
H3K9me3
KDM4A
KDM4B
KDM4C
background
0.5
1.5145
0.5
5.4
3.4
5.4
ABCF1
PPP1R10
MRPS18B
ATAT1

FIG. 21

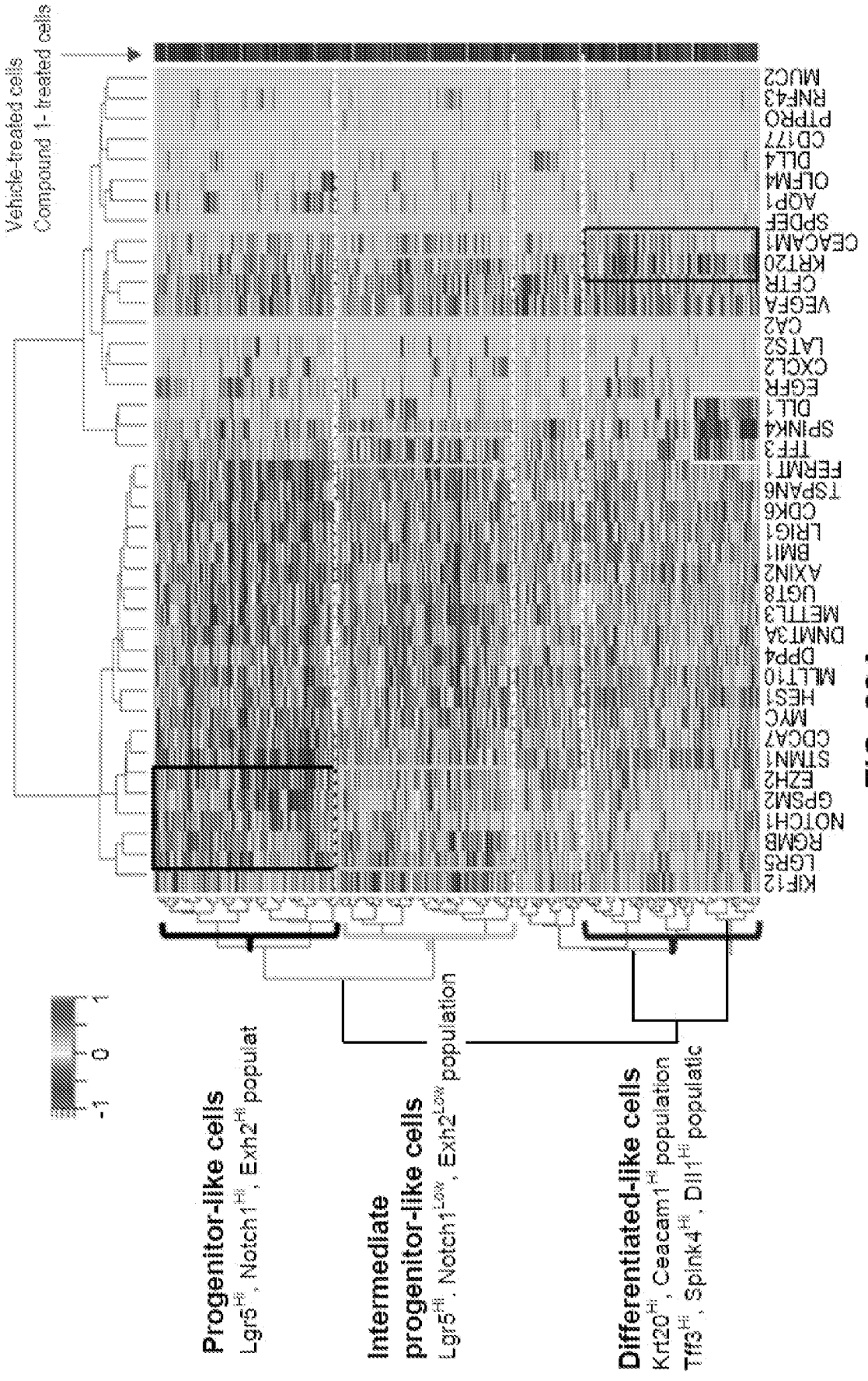

Vehicle-treated cells
Compound 1- treated cells
KIF12
LGR5
RGMB
NOTCH1
GPSM2
EZH2
STMN1
CDCA7
MYC
HES1
MLLT10
DPP4
DNMT3A
METTL3
UGT8
AXIN2
BMI1
LRIG1
CDK6
TSPAN6
FERMT1
TFF3
SPINK4
DLL1
EGFR
CXCL2
LATS2
CA2
VEGFA
CFTR
KRT20
CEACAM1
SPDEF
AQP1
OLFM4
DLL4
CD177
PTPRO
RNF43
MUC2
-1
0
1
Progenitor-like cells
Lgr5Hi, Notch1Hi, Exh2Hi populat
Intermediate progenitor-like cells
Lgr5Hi, Notch1Low, Exh2Low population
Differentiated-like cells
Krt20Hi, Ceacam1Hi population
Tff3Hi, Spink4Hi, Dll1Hi populatio

FIG. 22A

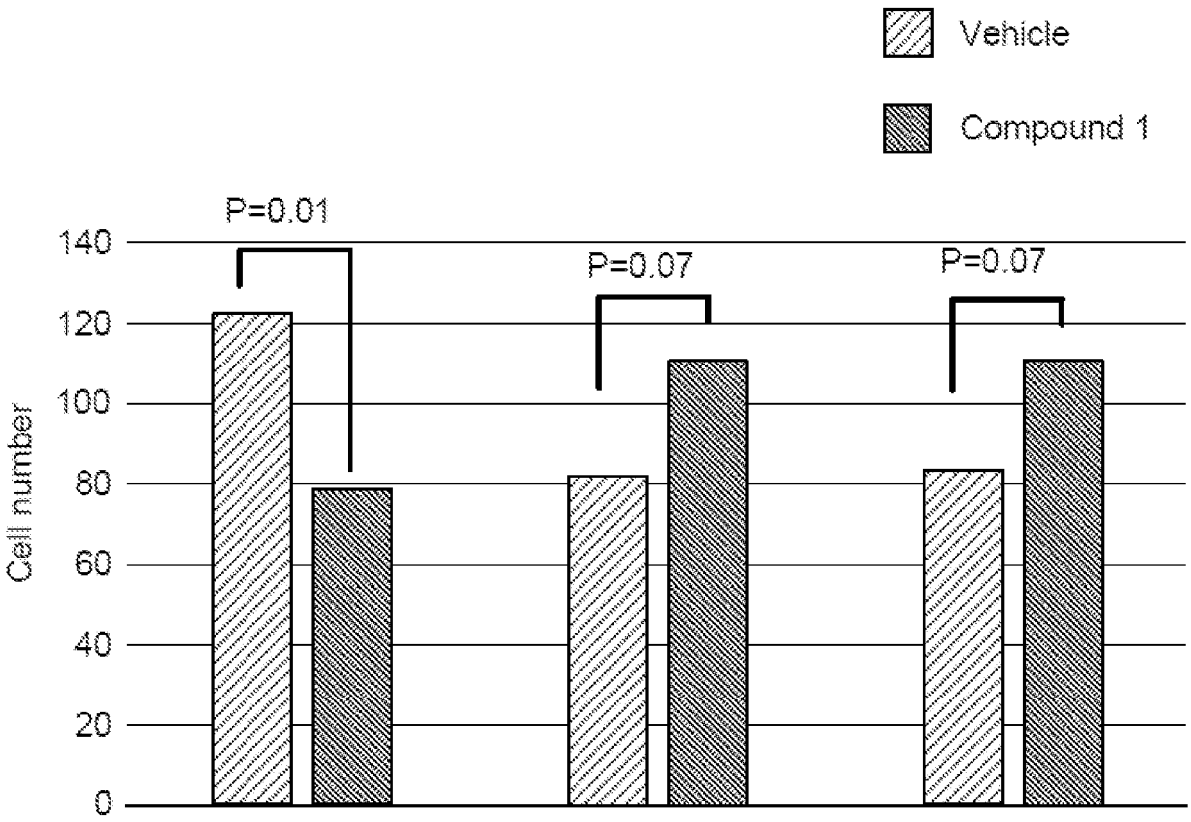

Vehicle
Compound 1
P=0.01
P=0.07
P=0.07
Cell number
140
120
100
80
60
40
20
0

FIG. 22B