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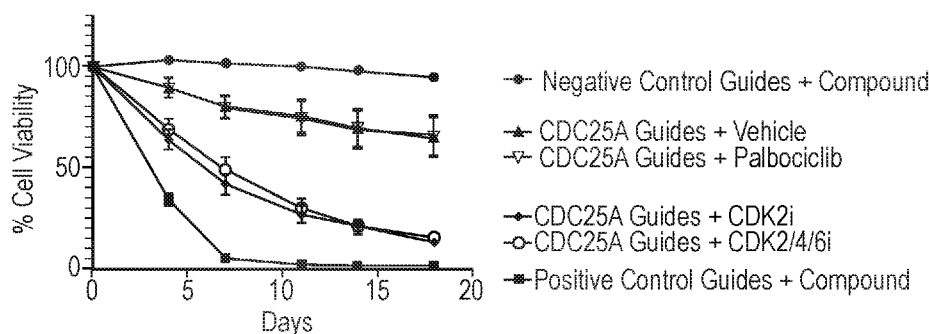
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(54) Title: COMBINATION THERAPIES USING CDK2 AND CDC25A INHIBITORS

FIG. 3B

CDC25A Drop Out Validation in H1048



CDK2i: 150 nM
 CDK2/4/6i: 50 nM
 Palbociclib (CDK4/6i): 150 nM

(57) Abstract: This invention relates to a method for treating cancer by administering an amount of a cyclin dependent kinase 2 (CDK2) inhibitor in combination with an amount of a cell division cycle 25A (CDC25A) inhibitor, wherein the amounts together are effective in treating cancer. This invention further relates to associated combinations, pharmaceutical compositions and uses thereof.

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COMBINATION THERAPIES USING CDK2 AND CDC25A INHIBITORS

REFERENCE TO SEQUENCE LISTING

This application is being filed electronically *via* EFSWeb and includes an electronically submitted sequence listing in .txt format. The .txt file contains a sequence listing entitled "PC072581A_SEQLISTING_ST25.txt" created on July 20, 2021 and having a size of 26 KB. The sequence listing contained in this .txt file is part of the specification and is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to combination therapies useful for the treatment of cancers. In particular, the present invention relates to combination therapies which comprise administering a CDK inhibitor, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising such compounds or salts, in combination with a CDC25A inhibitor, or a pharmaceutically acceptable salt thereof. The present invention also relates to associated methods of treatment, pharmaceutical compositions, and pharmaceutical uses. The methods and compositions are useful for any indication for which the therapeutic is itself useful in the detection, treatment and/or prevention of a disease, disorder, or other condition of a subject.

BACKGROUND

Cyclin dependent kinases (CDKs) are important cellular enzymes that perform essential functions in regulating eukaryotic cell division and proliferation. Therefore, CDK inhibitors are therapies useful in the treatment of cancer. The cyclin dependent kinase catalytic units are activated by regulatory subunits known as cyclins. At least sixteen mammalian cyclins have been identified (Johnson *et. al.*, Cyclins and Cell Cycle Checkpoints, *Annu. Rev. Pharmacol. Toxicol.* 1999, 39:295312). Cyclin B/CDK1, cyclin A/CDK2, cyclin E/CDK2, cyclin D/CDK4, cyclin D/CDK6, and likely other heterodynes are important regulators of cell cycle progression. Additional functions of cyclin/CDK heterodynes include regulation of transcription, DNA repair, differentiation and apoptosis (Morgan DO., Cyclin dependent kinases: engines, clocks, and microprocessors, *Annu. Rev. Cell. Dev. Biol.* 1997, 13:261291).

Cyclin dependent kinase inhibitors have been demonstrated to be useful in treating cancer. Increased activity or temporally abnormal activation of cyclin dependent kinases has been shown to result in the development of human tumors, and human tumor

development is commonly associated with alterations in either the CDK proteins themselves or their regulators (Cordon Cardio C., Mutations of cell cycle regulators: biological and clinical implications for human neoplasia, *Am. J. Pathol.* 1995, 147:545560; Karp *et al.*, Molecular foundations of cancer: new targets for intervention, *Nat. Med.* 1995, 1:309320; Hall *et al.*, Genetic alterations of cyclins, cyclin dependent kinases, and CDK inhibitors in human cancer, *Adv. Cancer Res.* 1996, 68:67108). Amplifications of the regulatory subunits of CDKs and cyclins, and mutation, gene deletion, or transcriptional silencing of endogenous CDK inhibitors have also been reported (Smalley *et al.*, Identification of a novel subgroup of melanomas with KIT/cyclin dependent kinase4 overexpression, *Cancer Res.* 2008, 68: 574352).

Overexpression of CDK2 is associated with abnormal regulation of cell-cycle. The cyclin E/CDK2 complex plays an important role in regulation of the G1/S transition, histone biosynthesis and centrosome duplication. Progressive phosphorylation of retinoblastoma (RB) protein (a negative regulator and tumor suppressor) by cyclin D/CDK4/6 and cyclin E/CDK2 releases the G1 transcription factor, E2F, and promotes S-phase entry. Activation of cyclin A/CDK2 during early S-phase promotes phosphorylation of endogenous substrates that permit DNA replication and inactivation of E2F, for S-phase completion (Asghar *et al.*, The history and future of targeting cyclin-dependent kinases in cancer therapy, *Nat. Rev. Drug. Discov.* 2015, 14(2): 130-146).

Cyclin E, the regulatory cyclin for CDK2, is frequently overexpressed in cancer. Cyclin E amplification or overexpression has long been associated with poor outcomes in breast cancer (Keyomarsi *et al.*, Cyclin E and survival in patients with breast cancer, *N Engl J Med.* 2002, 347:1566-75). Cyclin E2 (CCNE2) overexpression is associated with endocrine resistance in breast cancer cells and CDK2 inhibition has been reported to restore sensitivity to tamoxifen or CDK4 inhibitors in tamoxifen-resistant and CCNE2 overexpressing cells (Caldon *et al.*, Cyclin E2 overexpression is associated with endocrine resistance but not insensitivity to CDK2 inhibition in human breast cancer cells. *Mol Cancer Ther.* 2012, 11:1488-99; Herrera-Abreu *et al.*, Early Adaptation and Acquired Resistance to CDK4/6 Inhibition in Estrogen Receptor–Positive Breast Cancer, *Cancer Res.* 2016, 76: 2301–2313). Cyclin E amplification also reportedly contributes to trastuzumab resistance in HER2+ breast cancer (Scaltriti *et al.* Cyclin E amplification/overexpression is a mechanism of trastuzumab resistance in HER2+ breast cancer patients, *Proc Natl Acad Sci.* 2011, 108: 3761-6). Cyclin E overexpression has also been reported to play a role in basal-like and triple negative breast cancer (TNBC), as well as inflammatory breast cancer (Elsawaf & Sinn, Triple Negative Breast Cancer:

Clinical and Histological Correlations, *Breast Care* 2011, 6:273-278; Alexander *et al.*, Cyclin E overexpression as a biomarker for combination treatment strategies in inflammatory breast cancer, *Oncotarget* 2017, 8: 14897-14911).

CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib have been approved for treatment of hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced or metastatic breast cancer in combination with aromatase inhibitors in post-menopausal women, and in combination with fulvestrant after disease progression following endocrine therapy, (O'Leary *et al.*, Treating cancer with selective CDK4/6 inhibitors. *Nature Reviews* 2016, 13:417-430). While CDK4/6 inhibitors have shown significant clinical efficacy in HR-positive metastatic breast cancer, as with other kinases their effects may be limited over time by the development of primary or acquired resistance.

Amplification or overexpression of cyclin E1 (CCNE1) is associated with poor outcomes in ovarian, gastric, endometrial and other cancers (Nakayama *et al.*, Gene amplification CCNE1 is related to poor survival and potential therapeutic target in ovarian cancer, *Cancer* 2010, 116: 2621-34; Etemadmoghadam *et al.*, Resistance to CDK2 Inhibitors Is Associated with Selection of Polyploid Cells in CCNE1-Amplified Ovarian Cancer, *Clin. Cancer Res.* 2013, 19: 5960-71; Au-Yeung *et al.*, Selective Targeting of Cyclin E1-Amplified High-Grade Serous Ovarian Cancer by Cyclin-Dependent Kinase 2 and AKT Inhibition, *Clin. Cancer Res.* 2017, 23:1862-1874; Ayhan *et al.*, CCNE1 copy-number gain and overexpression identify ovarian clear cell carcinoma with a poor prognosis, *Modern Pathology* 2017, 30: 297-303; Ooi *et al.*, Gene amplification of CCNE1, CCND1, and CDK6 in gastric cancers detected by multiplex ligation-dependent probe amplification and fluorescence in situ hybridization, *Hum Pathol.* 2017, 61: 58-67; Noske *et. al.*, Detection of CCNE1/URI (19q12) amplification by in situ hybridization is common in high grade and type II endometrial cancer, *Oncotarget*, 2017, 8:14794-14805, Noske, *et. al.*, Detection of CCNE1/URI (19q12) amplification by in situ hybridisation is common in high grade and type II endometrial cancer, *Oncotarget* 2017, 8: 14794-14805).

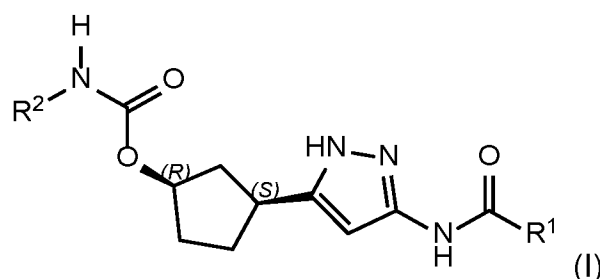
Improved combination therapies for the treatment of cancers, including methods of reducing resistance to such cancer therapeutics, comprise a large unmet medical need and the identification of novel combination regimens are required to improve treatment outcome.

SUMMARY OF THE INVENTION

Described herein are methods, combinations, pharmaceutical compositions, uses and kits related to the treatment of a disease or disorder, such as cancer, comprising administering a combination of a CDK2 inhibitor and a CDC25A inhibitor.

In one aspect, the present invention provides a method for treating cancer comprising administering to a subject in need thereof, an amount of a cyclin dependent kinase 2 (CDK2) inhibitor in combination with an amount of a cell division cycle 25A (CDC25A) inhibitor, wherein the amounts together are effective in treating cancer.

In certain embodiments of the methods of the present invention, the CDK2 inhibitor is a compound of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

R¹ is -L-(5-6 membered heteroaryl) or -L-(phenyl), where said 5-6 membered heteroaryl or phenyl is optionally substituted by one to three R³;

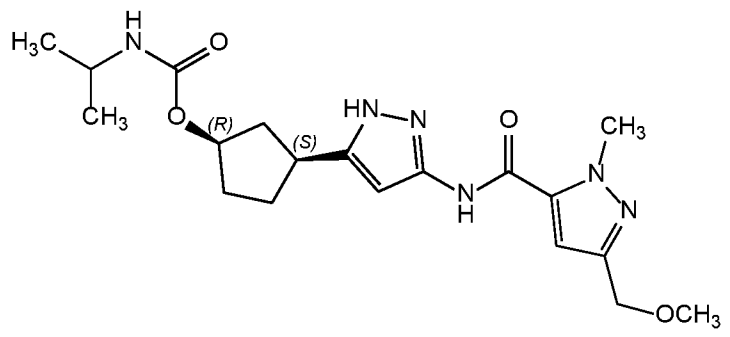
R² is C₁-C₆ alkyl or C₃-C₇ cycloalkyl, where said C₃-C₇ cycloalkyl is optionally substituted by C₁-C₄ alkyl;

L is a bond or a methylene; and

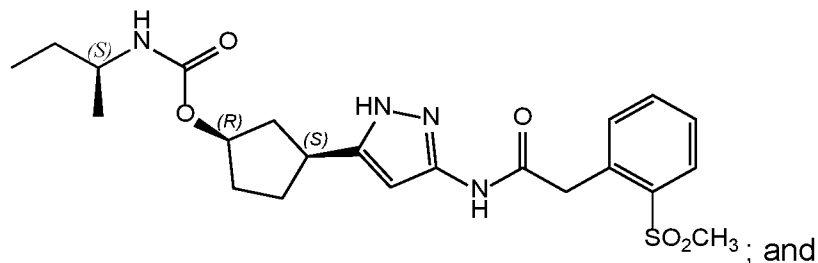
each R³ is independently C₁-C₄ alkyl, C₁-C₄ alkoxy or SO₂-C₁-C₄ alkyl, where each C₁-C₄ alkyl is optionally substituted by F, OH or C₁-C₄ alkoxy.

In certain embodiments of the methods of the present invention, the CDK2 inhibitor is selected from the group consisting of:

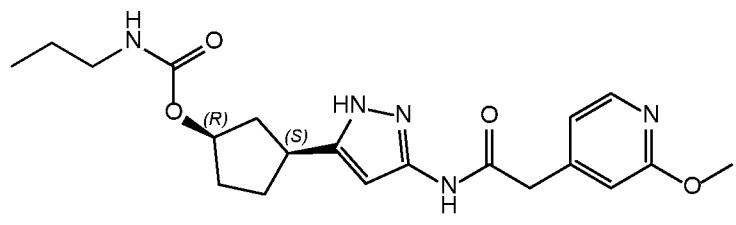
(1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A), having the structure:



(1*R*,3*S*)-3-[3-({[2-(methylsulfonyl)phenyl]acetyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate (COMPOUND B), having the structure:

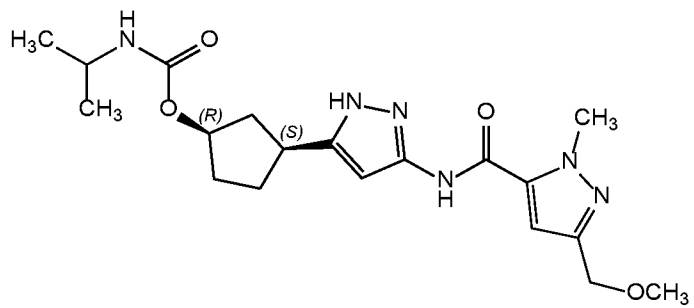


(1*R*,3*S*)-3-(3-{{(2-methoxypyridin-4-yl)acetyl}amino}-1*H*-pyrazol-5-yl)cyclopentyl propylcarbamate (COMPOUND C), having the structure:

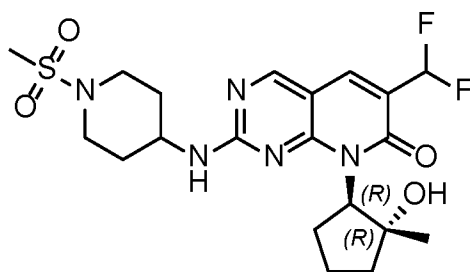


or a pharmaceutically acceptable salt thereof.

In certain embodiments of the methods of the present invention, the CDK2 inhibitor is (1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A), having the structure:



In certain embodiments of the methods of the present invention, the CDK2 inhibitor is 6-(difluoromethyl)-8-((1*R*,2*R*)-2-hydroxy-2-methylcyclopentyl)-2-(1-(methylsulfonyl)piperidin-4-ylamino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF-06873600), having the structure:



or a pharmaceutically acceptable salt thereof.

In certain embodiments of the methods of the present invention, the CDC25A inhibitor comprises a small molecule inhibitor, a small molecule degrader, a polypeptide, a peptide, a glycoprotein, a peptidomimetic, an antigen binding construct, a nucleic acid, a genetic construct for targeted gene editing, an antibody-like protein scaffold, an aptamer, or a combination thereof.

In certain embodiments of the methods of the present invention, the nucleic acid is small interfering RNA (siRNA), short hairpin RNA (shRNA), antisense or inhibitory DNA or RNA, ribozyme, RNA or DNA aptamer, RNAi, or peptide nucleic acid (PNA), or a combination thereof.

In certain embodiments of the methods of the present invention, the genetic construct for targeted gene editing is CRISPR/Cas9 construct, guide RNA (gRNA), guide DNA (gDNA) or tracrRNA, or a combination thereof.

In certain embodiments of the methods of the present invention, the polypeptide is an antibody or antibody fragment thereof.

In certain embodiments of the methods of the present invention, the small molecule inhibitor is 2-(2-mercaptoethanol)-3-methyl-1,4-naphthoquinone (synthetic vitamin K analog Cpd5), phenyl maleimide compound 1-([1,1'-biphenyl]-4-yl)-3,4-bis((2-hydroxyethyl)thio)-1*H*-pyrrole-2,5-dione (PM-20), quinone compound 2-(2,5-difluorophenyl)-6-((3-(methyl(3-((2-methyl-4,7-dioxo-4,7-dihydrobenzo[*d*]thiazol-5-yl)amino)propyl)amino)propyl)amino)benzo[*d*]oxazole-4,7-dione (IRC 083864), or 2-methoxyestadiol, or a pharmaceutically acceptable salt thereof.

In a preferred embodiment, the present invention provides a method for treating cancer comprising administering to a subject in need thereof, an amount of (1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A), in combination with amount of a CDC25A inhibitor, wherein the amounts together are effective in treating cancer.

In yet another embodiment, the present invention provides a method for treating cancer comprising administering to a subject in need thereof, an amount of (1*R*,3*S*)-3-[3-({[2-(methylsulfonyl)phenyl]acetyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate (COMPOUND B), in combination with amount of a CDC25A inhibitor, wherein the amounts together are effective in treating cancer.

In yet another embodiment, the present invention provides a method for treating cancer comprising administering to a subject in need thereof an amount (1*R*,3*S*)-3-(3-{{[2-methoxypyridin-4-yl]acetyl}amino}-1*H*-pyrazol-5-yl)cyclopentyl propylcarbamate

(COMPOUND C), in combination with an amount of a CDC25A inhibitor, wherein the amounts together are effective in treating cancer.

In another preferred embodiment, the present invention provides a method for treating cancer comprising administering to a subject in need thereof, an amount of 6-
5 (difluoromethyl)-8-((1R,2R)-2-hydroxy-2-methylcyclopentyl)-2-(1-(methylsulfonyl)piperidin-4-ylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (PF-06873600), or a pharmaceutically acceptable salt thereof, in combination with an amount of a CDC25A inhibitor, wherein the amounts together are effective in treating cancer.

In some embodiments of each of the methods herein, the method further
10 comprises administering an amount of an additional anti-cancer agent, in combination with the CDK2 inhibitor and the CDC25A inhibitor, wherein the amounts together are effective in treating cancer.

In some embodiments of the methods of the present invention, the subject is a human.

In certain embodiments of the methods of the present invention, the CDK2 inhibitor and the CDC25A inhibitor are administered simultaneously, sequentially or separately.
15 In some such embodiments, the CDK2 inhibitor and the CDC25A inhibitor are each administered in a therapeutic amount with respect to each other. In some such embodiments, the CDK2 inhibitor and the CDC25A inhibitor are each administered in a
20 sub-therapeutic amount with respect to each other.

Additional embodiments described herein relate to a combination comprising: a.
(i) (1R,3S)-3-[3-({[3-(methoxymethyl)-1-methyl-1H-pyrazol-5-yl]carbonyl}amino)-1H-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A) and (ii) a CDC25A inhibitor; b. (i) (1R,3S)-3-[3-({[2-(methylsulfonyl)phenyl]acetyl}amino)-1H-pyrazol-5-yl]cyclopentyl (2S)-butan-2-ylcarbamate (COMPOUND B), and (ii) a CDC25A inhibitor; c.
25 (i) (1R,3S)-3-(3-{{[2-methoxypyridin-4-yl]acetyl}amino}-1H-pyrazol-5-yl)cyclopentyl propylcarbamate (COMPOUND C), and (ii) a CDC25A inhibitor; or d. (i) 6-(difluoromethyl)-8-((1R,2R)-2-hydroxy-2-methylcyclopentyl)-2-(1-(methylsulfonyl)piperidin-4-ylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (PF-06873600), or
30 a pharmaceutically acceptable salt thereof; and (ii) a CDC25A inhibitor; for use in the treatment of cancer in a subject.

In some embodiments, the combination of the present invention is synergistic. In certain embodiments of the combination of the present invention, the subject is a human.

In one aspect, the present invention provides a pharmaceutical composition
35 comprising a CDK2 inhibitor and a pharmaceutical composition comprising a CDC25A

inhibitor, for simultaneous, sequential or separate use in therapy, and a pharmaceutically acceptable carrier.

Additional embodiments described herein relate to a separate or sequential use in therapy, wherein the pharmaceutical composition comprising a CDK2 inhibitor and the pharmaceutical composition comprising a CDC25A inhibitor are administered sequentially.

In certain embodiments of the present invention, the pharmaceutical composition of the CDK2 inhibitor is selected from the group consisting of: a. (1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A); b. (1*R*,3*S*)-3-[3-({[2-(methylsulfonyl)phenyl]acetyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate (COMPOUND B); c. ((1*R*,3*S*)-3-(3-{{[2-methoxypyridin-4-yl]acetyl}amino}-1*H*-pyrazol-5-yl)cyclopentyl propylcarbamate (COMPOUND C); and d. 6-(difluoromethyl)-8-((1*R*,2*R*)-2-hydroxy-2-methylcyclopentyl)-2-(1-(methylsulfonyl)piperidin-4-ylamino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF-06873600), or a pharmaceutically acceptable salt thereof.

Additional embodiments described herein relate to a pharmaceutical composition for use in the treatment of cancer.

Some embodiments described herein relate to a use of a combination comprising a CDK2 inhibitor and a CDC25A inhibitor for treating cancer in a subject.

Some embodiments described herein relate to a use of a CDK2 inhibitor and a CDC25A inhibitor in the preparation of a medicament for treating cancer in a subject.

Some embodiments described herein relate to a use of a CDK2 inhibitor in the preparation of a medicament for the treatment of cancer, wherein the medicament is for use in combination therapy with a CDC25A inhibitor.

Some embodiments described herein relate to a use of a CDC25A inhibitor in the preparation of a medicament for the treatment of cancer, wherein the medicament is for use in combination therapy with a CDK2 inhibitor.

In certain embodiments of the use of the present invention, the CDK2 inhibitor is selected from the group consisting of: a. (1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A); b. (1*R*,3*S*)-3-[3-({[2-(methylsulfonyl)phenyl]acetyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate (COMPOUND B); c. (1*R*,3*S*)-3-(3-{{[2-methoxypyridin-4-yl]acetyl}amino}-1*H*-pyrazol-5-yl)cyclopentyl propylcarbamate (COMPOUND C); and d. 6-(difluoromethyl)-8-((1*R*,2*R*)-2-hydroxy-2-methylcyclopentyl)-

2-(1-(methylsulfonyl)piperidin-4-ylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (PF-06873600), or a pharmaceutically acceptable salt thereof.

In some such embodiments, the combination is a synergistic combination.

In some embodiments of each of the methods, combinations, pharmaceutical compositions and uses of the present invention, the cancer is a solid tumor cancer. In some such embodiments, the cancer is a hematologic cancer. In some such embodiments, the cancer is a cyclin E dominant cancer.

Examples of solid tumor cancers include, but are not limited to, lung cancer (including small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC)), breast cancer, brain cancer, head and neck cancer (including squamous cell carcinoma of the head and neck (SCCHN)), prostate cancer (including neuroendocrine prostate cancer (NEPC)), ovarian cancer, bladder cancer, bone cancer, colorectal cancer, kidney cancer, liver cancer (including hepatocellular carcinoma (HCC)), stomach cancer, pancreatic cancer, esophageal cancer, cervical cancer, sarcoma, skin cancer (including squamous cell carcinoma), mesothelioma, malignant rhabdoid tumors, neuroblastoma, and diffuse intrinsic pontine glioma (DIPG).

In some embodiments, the cancer is selected from the group consisting of lung cancer, breast cancer, brain cancer, head and neck cancer, prostate cancer, ovarian cancer, bladder cancer, bone cancer, colorectal cancer, kidney cancer, liver cancer, stomach cancer, pancreatic cancer, esophageal cancer, cervical cancer, sarcoma, skin cancer, mesothelioma, malignant rhabdoid tumors, neuroblastoma, and diffuse intrinsic pontine glioma (DIPG).

Examples of hematologic cancers include, but are not limited to, acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), small lymphocytic lymphoma, hairy cell leukemia, chronic myelomonocytic leukemia (CMML), adult T-cell leukemia/lymphoma (ATLL), Hodgkin's lymphoma, non-Hodgkin's lymphoma (NHL), multiple myeloma, plasmacytoma, plasma cell leukemia, and amyloidosis.

Examples of cyclin E dominant cancers include, but are not limited to, ovarian cancer, breast cancer, prostate cancer, small cell lung cancer, colorectal cancer or pancreatic cancer.

Further embodiments described herein relate to a kit comprising: (i) a pharmaceutical composition comprising a CDK2 inhibitor and a pharmaceutically acceptable carrier; and (ii) a pharmaceutical composition comprising a CDC25A and a pharmaceutically acceptable carrier; and instructions for dosing of the pharmaceutical

compositions for the treatment of cancer. In some such embodiments, the CDK2 inhibitor is selected from the group consisting of: a. (1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A); b. (1*R*,3*S*)-3-[3-({[2-(methylsulfonyl)phenyl]acetyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate (COMPOUND B); c. (1*R*,3*S*)-3-(3-({[(2-methoxypyridin-4-yl)acetyl]amino}-1*H*-pyrazol-5-yl)cyclopentyl propylcarbamate (COMPOUND C), or a pharmaceutically acceptable salt thereof; and d. 6-(difluoromethyl)-8-((1*R*,2*R*)-2-hydroxy-2-methylcyclopentyl)-2-(1-(methylsulfonyl)piperidin-4-ylamino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF-06873600), or a pharmaceutically acceptable salt thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows CRISPR screen workflow.

FIG. 2 shows synthetic lethal targets in three small cell lung cancer (SCLC) cell lines with CDK2 inhibition identified by CRISPR screens. FIG. 2A shows synthetic lethal targets in three SCLC cell lines with CDK2 inhibitors (CDK2i). FIG. 2B shows synthetic lethal targets in three SCLC with CDK2/4/6 inhibitor.

FIG. 3 shows sgRNA competition assays using the U6-sgRNA-EFS-GFP plasmids. FIG. 3A shows competition assay workflow. FIG. 3B shows validation of CDC25A as a synthetic lethal target with CDK2 inhibition by CRISPR knockout in SCLC cell model H1048 cells. Variabilities across different CDC25A sgRNAs represented by the error bar (SD) at individual time points. FIG. 3C shows validation of CDC25A as a synthetic lethal target with CDK2 inhibition by CRISPR knockout in SCLC cell model H1876 cells. Variabilities across different CDC25A sgRNAs represented by the error bar (SD) at individual time points. FIG. 3D shows validation of CDC25A as a synthetic lethal target with CDK2 inhibition by CRISPR knockout in SCLC cell model H211 cells. Variabilities across different CDC25A sgRNAs represented by the error bar (SD) at individual time points.

FIG. 4 shows validation of CDC25A as a synthetic lethal target with CDK2 but not CDK4/6 inhibition by shRNA knockdown in SCLC cell model H82 cells. Error bars represent SD for 3 replicates. FIG. 4A shows validation of CDC25A as a synthetic lethal target with CDK2 inhibition by shRNA knockdown in SCLC cell model H82 cells. FIG. 4B shows validation of CDC25A as a synthetic lethal target with CDK2/4/6 inhibition by shRNA knockdown in SCLC cell model H82 cells. Error bars represent SD for 3 replicates. FIG. 4C shows CDC25A knockdown by shRNA knockdown does not

synergize with CDK4/6 inhibition in SCLC cell model H82 cells. Error bars represent SD for 3 replicates.

DETAILED DESCRIPTION

The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the present invention and the Examples included herein. Each of the embodiments of the present invention described below may be combined with one or more other embodiments of the present invention described herein which is not inconsistent with the embodiment(s) with which it is combined. Reference throughout this specification to “one embodiment,” “an embodiment,” or “some embodiments,” means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention.

It is to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting. For example, each of the embodiments described herein envisions within its scope pharmaceutically acceptable salts of the small molecule compounds described herein. Accordingly, the phrase “or a pharmaceutically acceptable salt thereof” is implicit in the description of all small molecule compounds described herein. It is further to be understood that unless specifically defined herein, the terminology used herein is to be given its traditional meaning as known in the relevant art.

As used herein the singular forms, “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a cell” includes one or more cells and equivalents thereof known to those skilled in the art, and so forth. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. Hence “comprising A or B” means including A, or B, or A and B. Furthermore, the use of the term “including,” as well as other related forms, such as “includes” and “included,” is not limiting.

As used herein, the term “about” is a flexible word with a meaning similar to “approximately” or “nearly”. The term “about” indicates that exactitude is not claimed, but rather a contemplated variation. Thus, as used herein, the term “about” means within 1 or 2 standard deviations from the specifically recited value, or $\pm$ a range of up to 20%, up to 15%, up to 10%, up to 5%, or up to 4%, 3%, 2%, or 1 % compared to the specifically recited value.

As used herein, the term "SD" refers to the standard deviation using known statistical methods.

As used herein, the term "KD" is intended to refer to the dissociation constant, which is obtained from the ratio of Kd to Ka (*i.e.*, Kd/Ka) and is expressed as a molar concentration (M). KD values for binding biomolecules can be determined using methods well established in the art.

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

The terms "polypeptide," "protein," "peptide," "peptide sequence," "amino acid sequence," and "polypeptide sequence" are used interchangeably herein to refer to at least two amino acids or amino acid analogs which are covalently linked by a peptide bond or an analog of a peptide bond.

The term "nucleic acid," "polynucleotide," "nucleotide sequence," "nucleotide," "oligonucleotide," "oligomer," or "oligo" refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner *et al.*, Enhanced evolutionary PCR using oligonucleotides with inosine at the 3'-terminus, *Nucleic Acid Research*, 1991, 19:5081; Ohtsuka *et al.*, An alternative approach to deoxyoligonucleotides as hybridization probes by insertion of deoxyinosine at ambiguous codon positions, *J. Biol. Chem.* 1985, 260:2605-2608; and Rossolini *et al.*, Use of deoxyinosine-containing primers vs degenerate primers for polymerase chain reaction based on ambiguous sequence information, *Mol. Cell. Probes*, 1994, 8:91-98). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

As used herein, the term “gene” means the segment of DNA involved in producing a polypeptide chain. It may include regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

As used herein, the term “genome” refers to the total genetic information or hereditary material possessed by an organism (including viruses), i.e., the entire genetic complement of an organism or virus. The genome generally refers to all of the genetic material in an organism's chromosome (s), and in addition, extra chromosomal, genetic information that is stably transmitted to daughter cells (e.g., the mitochondrial genome).

A genome can comprise RNA or DNA.

As used herein, the term “knockdown” refers to a decrease in gene expression of one or more genes.

As used herein, the term “knockout” refers to the ablation of gene expression of one or more genes.

As used herein, the term “promoter” is defined as an array of nucleic acid control sequences that direct transcription of a nucleic acid. As used herein, a promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription.

"Clustered Regularly Interspaced Short Palindromic Repeats" and "CRISPRs", as used interchangeably herein refer to loci containing multiple short direct repeats that are found in the genomes of approximately 40% of sequenced bacteria and 90% of sequenced archaea. The CRISPR system is a microbial nuclease system involved in defense against invading phages and plasmids that provides a form of acquired immunity.

The CRISPR loci in microbial hosts contain a combination of CRISPR-associated (Cas) genes as well as non-coding RNA elements capable of programming the specificity of the CRISPR-mediated nucleic acid cleavage. Short segments of foreign DNA, called spacers, are incorporated into the genome between CRISPR repeats, and serve as a 'memory' of past exposures. Cas9 forms a complex with the 3' end of the single guide RNA (sgRNA), and the protein-RNA pair recognizes its genomic target by complementary base pairing between the 5' end of the sgRNA sequence and a predefined 20 bp DNA sequence, known as the protospacer. This complex is directed to homologous loci of pathogen DNA via regions encoded within the crRNA, i.e., the protospacers, and protospacer-adjacent motifs (PAMs) within the pathogen genome. The non-coding

CRISPR array is transcribed and cleaved within direct repeats into short crRNAs containing individual spacer sequences, which direct Cas nucleases to the target site (protospacer). By simply exchanging the 20 bp recognition sequence of the expressed sgRNA, the Cas9 nuclease can be directed to new genomic targets. CRISPR spacers are used to recognize and silence endogenous genetic elements. For example, the CRISPR spacer targeting CDC25A edits the CDC25A gene natural in the cells.

In certain embodiments, a PAM or PAM-like motif directs binding of the effector protein complex as disclosed herein to the target locus of interest. In some embodiments, the PAM may be a 5 PAM (*i.e.*, located upstream of the 5' end of the protospacer). In other embodiments, the PAM may be a 3 PAM (*i.e.*, located downstream of the 5' end of the protospacer). The term "PAM" may be used interchangeably with the term "PFS" or "protospacer flanking site" or "protospacer flanking sequence".

In some embodiments, a subject DNA-targeting RNA comprises two separate RNA molecules (RNA polynucleotides: an "activator-RNA" and a "targeter-RNA", see below) and is referred to herein as a "double-molecule DNA-targeting RNA" or a "two-molecule DNA-targeting RNA." In other embodiments, the subject DNA-targeting RNA is a single RNA molecule (single RNA polynucleotide) and is referred to herein as a "single-molecule DNA-targeting RNA," a "single-guide RNA," or an "sgRNA." The term "DNA-targeting RNA" or "gRNA" is inclusive, referring both to double-molecule DNA-targeting RNAs and to single-molecule DNA-targeting RNAs (*i.e.*, sgRNAs).

As used herein, a "CRISPR-Cas" or "CRISPR system," functional genomic as used in herein and in documents, such as WO 2014/093622 (PCT/US2013/074667), refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated (Cas) genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (*e.g.*, tracrRNA or an active partial tracrRNA), a tracr-mate sequence (encompassing a "direct repeat" and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a "spacer" in the context of an endogenous CRISPR system), or "RNA(s)" as that term is herein used (*e.g.*, RNA(s) to guide Cas, such as Cas9, *e.g.*, CRISPR RNA and transactivating (tracr) RNA or a single guide RNA (sgRNA) (chimeric RNA)) or other sequences and transcripts from a CRISPR locus. In general, a CRISPR system is characterized by elements that promote the formation of a CRISPR complex at the site of a target sequence (also referred to as a protospacer in the context of an endogenous CRISPR system), *e.g.*, Shmakov *et al.*, Discovery and Functional Characterization of

Diverse Class 2 CRISPR-Cas Systems, *Molecular Cell* 2015, DOI: dx.doi.org/10.1016/j.molcel.2015.10.008.

CRISPR/Cas systems include type I, II, and III sub-types. Wild-type type II CRISPR/Cas systems utilize the RNA-mediated nuclease, Cas9 in complex with guide and activating RNA to recognize and cleave foreign nucleic acid. Methods and compositions for controlling inhibition and/or activation of transcription of target genes, populations of target genes (e.g., controlling a transcriptome or portion thereof) are described, e.g., Gilbert *et al.*, Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation, *Cell* 2014; 159(3):647-61, the contents of which are incorporated by reference in the entirety for all purposes.

CRISPR-based methods are readily amenable to pooled screening experiments. Generally, a library of cells each with a specific genetic perturbation, such as deletion, knockdown, or overexpression of a gene, is exposed to a compound of interest, either in pooled or arrayed format. Quantification of the abundance of library members before and after treatment reveals the effect of each genetic perturbation on sensitivity. For example, deletion of a specific gene may confer hypersensitivity or resistance.

Cas9 homologs are found in a wide variety of eubacteria, including, but not limited to bacteria of the following taxonomic groups: Actinobacteria, Aquificae, Bacteroidetes-Chlorobi, Chlamydiae-Verrucomicrobia, Chloflexi, Cyanobacteria, Firmicutes, Proteobacteria, Spirochaetes, and Thermotogae. An exemplary Cas9 protein is the *Streptococcus pyogenes* Cas9 protein. Additional Cas9 proteins and homologs thereof are described in, e.g., Chylinski, *et al.*, The tracrRNA and Cas9 Families of Type II CRISPR-Cas Immunity Systems, *RNA Biol.* 2013,10(5): 726-737; Makarova *et al.*, Evolution and classification of the CRISPR-Cas systems, *Nat. Rev. Microbiol.* 2011, 9(6): 467-477; Hou, *et al.*, Efficient genome engineering in human pluripotent stem cells using Cas9 from *Neisseria meningitidis*, *Proc. Natl. Acad. Sci.* 2013, 110(39):15644-9; Sampson *et al.*, A CRISPR/Cas System Mediates Bacterial Innate Immune Evasion and Virulence, *Nature* 2013, 497(7448):254-7; and Jinek, *et al.*, A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity, *Science* 2012, 337(6096):816-21.

Because of its high efficiency and accuracy, the CRISPR-Cas9 functional genomic screening has recently emerged as a potentially powerful tool in drug target discovery. Among its many applications, CRISPR-Cas9 has shown an unprecedented clinical potential to discover novel targets for cancer therapy and to dissect chemical-genetic interactions, providing insight into how tumors respond to drug treatment.

CRISPR/Cas9 screens are platforms for oncology target discovery because they can uncover unique dependencies of oncogene addiction, lineage-specific regulators, synthetic lethal vulnerabilities of drug treatment, vulnerabilities of tumor immune evasion in genetically engineered mouse models, and physiologically relevant targets of the tumor microenvironment *in vivo* (Wang, *et. al.*, Genetic screens in human cells using the CRISPR-Cas9 system, *Science* 2014, 343, 80-84; Tsherniak, *et. al.*, Defining a Cancer Dependency Map, *Cell* 2017, 170, 564-576 e516; Chen, *et. al.*, Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis, *Cell* 2015, 160, 1246-1260; Manguso, *et. al.*, *In vivo* CRISPR screening identifies Ptpn2 as a cancer immunotherapy target, *Nature* 2017, 547, 413-418; Konermann, *et al.*, Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex, *Nature* 2015, 517, 583-588).

As used herein, "activity" in the context of CRISPR/Cas activity, Cas9 activity, sgRNA activity, sgRNA:nuclease activity and the like refers to the ability to bind to a target genetic element and/or modulate transcription at or near the target genetic element. Such activity can be measured in a variety of ways as known in the art. For example, expression, activity, or level of a reporter gene, or expression or activity of a gene encoded by the genetic element can be measured.

In one embodiment, the present invention employs the ProteIN ConsERvation (PINCER) genome-wide CRISPR library, which combines enzymatic efficiency optimization with conserved-length protein region targeting, and also incorporates domains, coding sequence position, U6 termination (TTT), restriction sites, polymorphisms, and specificity. In some such embodiments, the PINCER genome-wide CRISPR library combines Cas9 cleavage efficacy optimization with deletion-based protein conservation targeting.

Several aspects of the present invention relate to vector systems comprising one or more vectors, or vectors as such. Vectors can be designed for expression of CRISPR transcripts (e.g., nucleic acid transcripts, proteins, or enzymes) in prokaryotic or eukaryotic cells. For example, CRISPR transcripts can be expressed in bacterial cells such as *Escherichia coli*, yeast cells, or mammalian cells. Suitable host cells are discussed further in Keown, W. A., *et al.*, Methods for introducing DNA into mammalian cells, in *Methods in Enzymology 185: Gene Expression Technology* 1990 pp. 527–537, the contents of which are incorporated herein by reference. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example the lentiviral vectors encompassed in aspects of the present invention may comprise a U6 RNA pol III promoter.

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. Vectors include, but are not limited to, nucleic acid molecules that are single-stranded, double-stranded, or partially double-stranded; nucleic acid molecules that comprise one or more free ends, no free ends (e.g., circular); nucleic acid molecules that comprise DNA, RNA, or both; and other varieties of polynucleotides known in the art. A vector may be any of a number of nucleic acid molecules or viruses or portions thereof that are capable of mediating entry of, e.g., transferring, transporting, *etc.*, a nucleic acid of interest between different genetic environments or into a cell. The nucleic acid of interest may be linked to, e.g., inserted into, the vector using, e.g., restriction and ligation. Vectors include, for example, DNA or RNA plasmids, cosmids, naturally occurring or modified viral genomes or portions thereof, nucleic acids that can be packaged into viral capsids, mini-chromosomes, artificial chromosomes, *etc.*

One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be inserted, such as by standard molecular cloning techniques. Plasmid vectors typically include an origin of replication (e.g., for replication in prokaryotic cells). A plasmid may include part or all of a viral genome (e.g., a viral promoter, enhancer, processing or packaging signals, and/or sequences sufficient to give rise to a nucleic acid that can be integrated into the host cell genome and/or to give rise to infectious virus). Viruses or portions thereof that can be used to introduce nucleic acids into cells may be referred to as viral vectors.

Another type of vector is a viral vector, wherein virally-derived DNA or RNA sequences are present in the vector for packaging into a virus (e.g., retroviruses, replication defective retroviruses, adenoviruses, replication defective adenoviruses, and adeno-associated viruses).

In one embodiment, the vector transports the nucleic acid to cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. In general, the vectors useful in the present invention include, but are not limited to, plasmids, phagemids, viruses, or vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the antisense oligonucleotide siRNA or ribozyme nucleic acid sequences. In a particular embodiment, the viral vectors include, but are not limited to nucleic acid sequences from the following viruses: retrovirus (e.g., moloney murine leukemia virus, harvey murine sarcoma virus, murine mammary tumor virus, and rouse sarcoma virus) adenovirus, adeno-associated virus, SV40-type viruses, polyoma viruses, Epstein-Barr viruses, papilloma viruses, herpes virus, vaccinia

virus, polio virus, and RNA virus such as a retrovirus. One may employ other vectors not named but moieties known to those skilled in the art.

In some embodiments, the viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses (e.g., lentivirus), the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, M., *Gene Transfer and Expression A Laboratory Manual*, Pub. by *W. H. Freeman and Company*, 1990 pp 3-81, and in Murray, E.J., *et al.*, *Methods in Molecular Biology*, Vol. 7: *Gene Transfer and Expression Techniques*, *Humana Press Inc.* 1991 pp 109-128 .

As used herein, the term "lentivirus" refers to a genus of the retroviridae family. Lentiviruses are unique among the retroviruses in being able to infect non-dividing cells; they can deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector. HIV, SIV, and FIV are all examples of lentiviruses. Vectors derived from lentiviruses offer the means to achieve significant levels of gene transfer *in vivo*.

In some embodiments, viruses for certain applications are the adeno-viruses and adeno-associated viruses, which are double-stranded DNA viruses that have already been approved for human use in gene therapy. The adeno-associated virus may be engineered to be replication deficient and is capable of infecting a wide range of cell types and species.

Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors." Common expression vectors of utility in recombinant DNA techniques are often in the form of plasmids.

Some methods of the present invention can include inducing expression. In some methods of the present invention, the organism or subject is a eukaryote (including mammal or human) or a non-human eukaryote or a non-human animal or a non-human mammal. In some methods of the present invention the viral vector is a lentivirus-derived vector.

As used herein, the term "host cell" refers to cells that have been engineered to contain the modified sgRNA disclosed herein, and include archeal, prokaryotic, or eukaryotic. Thus, engineered, or recombinant cells, are distinguishable from naturally occurring cells that do not contain recombinantly introduced genes through recombinant techniques.

Methods for transforming a host cell with an expression vector may differ depending upon the species of the desired host cell. For example, yeast cells may be transformed by lithium acetate treatment (which may further include carrier DNA and PEG treatment) or electroporation. These methods are included for illustrative purposes and are in no way intended to be limiting or comprehensive. Routine experimentation through means well known in the art may be used to determine whether a particular expression vector or transformation method is suited for a given host cell. Furthermore, reagents and vectors suitable for many different host microorganisms are commercially available and/or well known in the art.

In some methods of the present invention, the CRISPR enzyme is a Cas9. In some methods of the present invention the CRISPR enzyme comprises one or more mutations in one of the catalytic domains. In some methods of the present invention the CRISPR enzyme is a Cas9 nickase. In some methods of the present invention the expression of the guide sequence is under the control of the T7 promoter that is driven by the expression of T7 polymerase. In some methods of the present invention the expression of the guide sequence is under the control of a U6 promoter.

In some embodiments, sgRNA expression vectors were constructed. In some such embodiments, the vector is configured to be conditional, whereby the vector targets only certain cell types. The vector may be a viral vector. The vector may be conditional by using a regulatory element that is cell or tissue specific. The regulatory element may be a promoter. The vector may be conditional by using a viral vector that infects a specific cell type. The vector may be any virus that efficiently targets cells of the central nervous system and does not illicit a strong immune reaction. The viral vector may be a lentivirus, an adenovirus, or an adeno associated virus (AAV). The virus envelope proteins may be chosen to cause the virus to have tropism towards a specific cell type.

The vector(s) can comprise Cas encoding sequences, and/or a single, but possibly also can comprise at least 3 or 8 or 16 or 32 or 48 or 50 guide RNA(s) (e.g., sgRNAs) encoding sequences, such as 1-2, 1-3, 1-4 1-5, 3-6, 3-7, 3-8, 3-9, 3-10, 3-8, 3-16, 3-30, 3-32, 3-48, 3-50 RNA(s) (e.g., sgRNAs). In a single vector there can be a promoter for each RNA (e.g., sgRNA), advantageously when there are up to about 16 RNA(s); and,

when a single vector provides for more than 16 RNA(s), one or more promoter(s) can drive expression of more than one of the RNA(s), *e.g.*, when there are 32 RNA(s), each promoter can drive expression of two RNA(s), and when there are 48 RNA(s), each promoter can drive expression of three RNA(s). By simple arithmetic and well-established cloning protocols and the teachings in this disclosure one skilled in the art can readily practice the present invention as to the RNA(s) for a suitable exemplary vector such as AAV, and a suitable promoter such as the U6 promoter. For example, the packaging limit of AAV is ~4.7 kb. The length of a single U6-gRNA (plus restriction sites for cloning) is 361 bp. Therefore, the skilled person can readily fit about 12-16, *e.g.*, 13 U6-gRNA cassettes in a single vector. This can be assembled by any suitable means, such as a golden gate strategy used for TALE assembly (genome-engineering.org/taleffectors/). The skilled person can also use a tandem guide strategy to increase the number of U6-gRNAs by approximately 1.5 times, *e.g.*, to increase from 12-16, *e.g.*, 13 to approximately 18-24, *e.g.*, about 19 U6-gRNAs. Therefore, one skilled in the art can readily reach approximately 18-24, *e.g.*, about 19 promoter-RNAs, *e.g.*, U6-gRNAs in a single vector, *e.g.*, an AAV vector. A further means for increasing the number of promoters and RNAs in a vector is to use a single promoter (*e.g.*, U6) to express an array of RNAs separated by cleavable sequences. And an even further means for increasing the number of promoter-RNAs in a vector, is to express an array of promoter-RNAs separated by cleavable sequences in the intron of a coding sequence or gene; and, in this instance it is advantageous to use a polymerase II promoter, which can have increased expression and enable the transcription of long RNA in a tissue specific manner (see, *e.g.*, nar.oxfordjournals.org/content/34/7/e53.short and nature.com/mt/journal/vl6/n9/abs/mt2008144a.html).

As used herein, the term "inhibits," or "inhibition" refers to the decrease in active of a target protein product relative to the normal wild type level. Inhibition may result in a decrease in activity of a target enzyme, a CDK inhibitor, or a compensatory rebound phosphorylation by CDK4 and/or CDK6 in response to the inhibition of CDK2 by less than 10%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%.

As used herein, the term "viability" refers to the measure of survivability of a testable culture in the presence of one or more inhibitors. For example, this may relate to the IC₅₀ of a drug, which is the half maximal inhibitory concentration (IC₅₀), or 25% inhibitory concentration (IC₂₅) of the drug and is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function.

In cell line, the effects of gene on different samples are defined as the “beta” score or “beta” value, a measurement of gene selections similar to the “log fold change” in differential expression analysis. The values of “beta” scores can be estimated by maximizing the joint log-likelihood of observing all sgRNA read counts on all different samples. This can be implemented using an Expectation-Maximization (EM) algorithm.

“CDK inhibitor” means any compound or agent that inhibits the activity of one or more CDK proteins or CDK/cyclin kinase complexes. The compound or agent may inhibit CDK activity, such as phosphorylation, by direct or indirect interaction with a CDK protein or it may activity act to prevent expression of one or more CDK genes. In one preferred embodiment, a CDK inhibitor may be a small molecule inhibitor.

CDKs and related serine/threonine kinases are important cellular enzymes that perform essential functions in regulating cell division and proliferation. “CDK inhibitor” means any compound or agent that inhibits the activity of one or more CDK proteins or CDK/cyclin kinase complexes. The compound or agent may inhibit CDK activity, such as phosphorylation, by direct or indirect interaction with a CDK protein or it may activity act to prevent expression of one or more CDK genes.

As used herein, references to specific proteins (e.g., CDC25A) may include a polypeptide having a native amino acid sequence, as well as variants and modified forms regardless of their origin or mode of preparation. A protein that has a native amino acid sequence is a protein having the same amino acid sequence as obtained from nature (e.g., CDC25A). Such native sequence proteins may be isolated from nature or may be prepared using standard recombinant and/or synthetic methods. Native sequence proteins specifically encompass naturally occurring truncated or soluble forms, naturally occurring variant forms (e.g., alternatively spliced forms), naturally occurring allelic variants and forms including post-translational modifications. A native sequence protein includes proteins following post-translational modifications such as glycosylation, or phosphorylation, or other modifications of some amino acid residues.

Variants refer to proteins that are functional equivalents to a native sequence protein that have similar amino acid sequences and retain, to some extent, one or more activities of the native protein. Variants also include fragments that retain activity. Variants also include proteins that are substantially identical (e.g., that have 80, 85, 90, 95, 97, 98, 99 percent, sequence identity) to a native sequence. Such variants include proteins having amino acid alterations such as deletions, insertions and/or substitutions. A “deletion” refers to the absence of one or more amino acid residues in the related protein.

The term "insertion" refers to the addition of one or more amino acids in the related protein.

A "substitution" refers to the replacement of one or more amino acid residues by another amino acid residue in the polypeptide. Typically, such alterations are conservative in nature such that the activity of the variant protein is substantially similar to a native sequence protein (see, e.g., (Creighton, T., *Proteins, Structures and Molecular Properties*, *W H Freeman*, 1984 pp. 55-60. In the case of substitutions, the amino acid replacing another amino acid usually has similar structural and/or chemical properties. Insertions and deletions are typically in the range of 1 to 5 amino acids, although depending upon the location of the insertion, more amino acids may be inserted or removed. The variations may be made using methods known in the art such as site-directed mutagenesis (Carter, *et al.*, Improved oligonucleotide site-directed mutagenesis using m13 vectors, *Nucl. Acids Res.* 1985, 13:4331; Zoller *et. al.* Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any fragment of DNA, *Nucl. Acids Res.*, 1982, 10:6487), cassette mutagenesis (Zoller, 1982), restriction selection mutagenesis (Wells *et al.*, Cassette Mutagenesis: An Efficient Method for Generation of Multiple Mutations at Defined Sites, *Gene* 1985 34:315-23), and PCR mutagenesis (Sambrook, *et al.*, *Molecular Cloning, A Laboratory Manual*, *Cold Spring Harbor Press* 1982 & 1989 2nd Edition, 2001 3rd Edition).

Two amino acid sequences are "substantially homologous" or "substantially similar" when greater than 80 percent, preferably greater than 85 percent, preferably greater than 90 percent of the amino acids are identical, or greater than about 90 percent, preferably greater than 95 percent, are similar (functionally identical). Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wis.) pileup program, or any of sequence comparison algorithms such as BLAST, FASTA, *etc.*

The term "expression" when used in the context of expression of a gene or nucleic acid refers to the conversion of the information, contained in a gene, into a gene product. A gene product may be the direct transcriptional product of a gene (e.g., mRNA, tRNA, rRNA, antisense RNA, ribozyme, structural RNA or any other type of RNA) or a protein produced by translation of a mRNA. Gene products also include messenger RNAs which are modified, by processes such as capping, polyadenylation, methylation, and editing, and proteins (e.g., EGFR) modified by, for example, methylation, acetylation,

phosphorylation, ubiquitination, SUMOylation, ADP-ribosylation, myristilation, and glycosylation.

An "inhibitor of expression" refers to a natural or synthetic compound that has a biological effect in inhibiting the expression of a gene.

As used herein, the term "CDC25 phosphatases" refers to protein phosphatases which belong, to the CDC25 family which are believed to be important regulators for the control of cell cycle progression by activating cyclin-dependent kinases (CDK). Three CDC25 homologs have been found in mammals: CDC25A, CDC25B, and CDC25C. Both CDC25B and CDC25C are thought to be regulators of G2/M transition through their ability to dephosphorylate and thus activate CDK1, a component of the CDK1/cyclin B mitotic kinase complex, which is required for cell entry into mitosis. CDC25A is likely to be important for G1/S phase transition by dephosphorylating and thus activating CDK2, 4, 6, which also form cyclin complexes, as well as in preserving genomic integrity, although CDC25A may also have some role in the initiation of mitosis. The CDC25A dephosphorylate CDK/cyclins on pThr14 and/or on pTyr15 residues.

CDC25 over-expression has been found in various human cancers and is correlated with a more aggressive disease and poor prognosis. CDC25B mRNA was first found to be over-expressed in cancer cell lines and SV40-transformed fibroblasts. Since then, CDC25A and CDC25B, but not CDC25C, have been found to be overexpressed in various cancer tissues, including those of breast, ovarian, prostate, lung, colorectal, esophageal, thyroid, laryngeal, hepatocellular, gastric, pancreatic, endometrial, head and neck, neuroblastoma, glioma, and lymphoma. As activators of the cell cycle-controlling CDKs, the CDC25 family of phosphatases are obvious-appreciated targets for anti-cancer therapy.

"Cell division cycle 25 A" or "CDC25A," a dual-specificity protein phosphatase, is one of the most crucial cell cycle regulators, which removes the inhibitory phosphorylation in cyclin-dependent kinases (CDKs), such as CDK2, CDK4, and CDK6, and positively regulates the activities of CDKs that lead to cell cycle progression. *In vitro* studies showed that in G1 phase, CDC25A is a positive regulator of CDK4 and CDK6. Later in G1 phase, CDC25A dephosphorylates the two inhibitory phosphorylation residues on CDK2 and activates it, promoting G1/S transition. In addition, CDC 25A also acts as a regulator of apoptosis. Overexpression of CDC25A promotes tumorigenesis and is frequently observed in various types of cancer (Ray D, Kiyokawa H., CDC25A phosphatase: a rate-limiting oncogene that determines genomic stability, *Cancer Res.* 2008, 68:1251–1253; Shen T, Huang S., The role of Cdc25A in the regulation of cell proliferation and

apoptosis, *Anticancer Agents Med Chem.* 2012, 12:631–639; Hoffmann, *et al.*, Activation of the phosphatase activity of human cdc25A by a cdk2-cyclin E dependent phosphorylation at the G1/S transition, *EMBO J.* 1994, 13:4302–4310).

Since CDC25A is a key component in stalling the cell cycle in response to DNA damage, identification of the specific combination can yield important tools in regulating CDC25A and thereby the cell cycle, as well as the cellular response to DNA damage.

As used herein, the term "CDC25A phosphatase inhibitor" or "CDC25A inhibitor," refers to any CDC25A phosphatase inhibitor that is currently known in the art or that will be identified in the future, and includes any chemical entity that, upon administration to a patient, results in inhibition of a biological activity associated with the CDC25A phosphatase in the patient (in particularly the dephosphorylation of CDK). Such CDC25A phosphatase inhibitor includes any agent (such as chemical entity and inhibitor of CDC25A expression) that blocks or inhibits CDC25A phosphatase activity. Such an inhibitor may act by binding directly to the CDC25A protein and inhibiting its phosphatase activity.

Examples of CDC25A phosphatase inhibitors include but are not limited to any of the CDC25A phosphatase inhibitors described in Lavecchia *et al.*, Inhibitors of Cdc25 Phosphatases as Anticancer Agents: A Patent Review, *Exp Opin. Ther Pat.* 2010 20(3):405-425, all of which are herein incorporated by reference.

As used herein, terms, including, but not limited to, "drug," "agent," "component," "composition," "compound," "substance," "targeted agent," "targeted therapeutic agent," "therapeutic agent," "pharmaceutical agent," and "medicament" may be used interchangeably to refer to the compounds of the present invention, *e.g.*, a CDK inhibitor, CDC25A inhibitors, or combinations thereof.

As used herein, the term "standard" refers to something used for comparison. For example, it can be a known standard agent or compound which is administered or added and used for comparing results when adding a test compound, or it can be a standard parameter or function which is measured to obtain a control value when measuring an effect of an agent or compound on a parameter or function.

As used herein, "modulate", "modulating", "modulation" and like terms refer to causing or facilitating a qualitative or quantitative change, alteration, or modification of a target, *e.g.*, activating (stimulating, upregulating) or inhibiting (suppressing, downregulating) a target. In some embodiment, "modulating" comprises increasing (enhancing) or decreasing (reducing) the amount or activity of a target. In various embodiments a "target" may be a gene, gene product, molecule, complex, biological

process, biological pathway, biological activity, biological process, chemical reaction, or a component of any of these. A "modulator" is an agent that modulates, and may be, e.g., an activator or an inhibitor.

Embodiments disclosed herein, comprise agents that are capable of activating or inhibiting the cell cycle. In some such embodiments, the agents of the present invention target the cell cycle progression regulators at the G1/S transition. In certain embodiments, the agent may inhibit or reduce signaling through CDK2/Cyclin E and CDK2/Cyclin A at the G1/S transition and in S-phase. In certain embodiments, the agent may be an inhibitor of CDC25A. In certain embodiments, the agent may be an inhibitor of CDK2 (CDK2 inhibitor or CDK2i). In certain embodiment, the agent may be a CDK2 inhibitor and a CDC25A inhibitor (CDC25A inhibitor or CDC25Ai). Embodiments disclosed herein may use more than one agent in combination and each agent may have the same or a different inhibitory effect.

An agent, as used herein, may or activate the expression of a gene, or the activity of a gene product. Exemplary agents include, but are not limited to: a small molecule compound (e.g., a CDK2 inhibitor or a CDC25 inhibitor), protein-binding agent that permits modulation of activity of proteins or disrupts interactions of proteins and other biomolecules (e.g., disrupting protein-protein interaction, ligand-receptor interaction, or protein nucleic acid interaction, an antibody or fragment thereof (e.g., an anti CDC25A antibody), a DNA targeting agent (e.g., CRISPR system, TALE, Zinc finger protein).

As used herein, the term "cytokine" refers generically to proteins released by one cell population that act on another cell as intercellular mediators or have an autocrine effect on the cells producing the proteins. Examples of such cytokines include lymphokines, monokines; interleukins ("ILs") such as IL- 1 , IL- 1a, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL10, IL-1 1 , IL-12, IL-13, IL-15, IL-17A-F, IL-18 to IL-29 (such as IL-23), IL-31, including PROLEUKIN® rIL-2; a tumor-necrosis factor such as TNF- α or TNF- β , TGF- β 1 -3; and other polypeptide factors including leukemia inhibitory factor ("LIF"), ciliary neurotrophic factor ("CNTF"), CNTF-like cytokine ("CLC"), cardiotrophin ("CT"), and kit ligand ("L").

As used herein, the term "chemokine" refers to soluble factors (e.g., cytokines) that have the ability to selectively induce chemotaxis and activation of leukocytes. They also trigger processes of angiogenesis, inflammation, wound healing, and tumorigenesis. Example chemokines include IL-8, a human homolog of murine keratinocyte chemoattractant (KC).

The terms “abnormal cell growth” and “hyperproliferative disorder” are used interchangeably in this application. “Abnormal cell growth,” as used herein, unless otherwise indicated, refers to cell growth that is independent of normal regulatory mechanisms (e.g., loss of contact inhibition). Abnormal cell growth may be benign (not
5 cancerous), or malignant (cancerous).

A “disorder” is any condition that would benefit from treatment with the compounds of the present invention. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the subject to the disorder in question.

As used herein, “metastasis” or “metastatic” is meant the spread of cancer from its
10 primary site to other places in the body. Cancer cells can break away from a primary tumor, penetrate into lymphatic and blood vessels, circulate through the bloodstream, and grow in a distant focus (metastasize) in normal tissues elsewhere in the body. Metastasis can be local or distant. Metastasis is a sequential process, contingent on tumor cells breaking off from the primary tumor, traveling through the bloodstream, and stopping at
15 a distant site. At the new site, the cells establish a blood supply and can grow to form a life-threatening mass. Both stimulatory and inhibitory molecular pathways within the tumor cell regulate this behavior, and interactions between the tumor cell and host cells in the distant site are also significant.

The term “cancer,” “cancerous,” or “malignant” refers to or describe the
20 physiological condition in subjects that is typically characterized by unregulated cell growth. The term “cancer” includes but is not limited to a primary cancer that originates at a specific site in the body, a metastatic cancer that has spread from the place in which it started to other parts of the body, a recurrence from the original primary cancer after remission, and a second primary cancer that is a new primary cancer in a person with a
25 history of previous cancer of a different type from the latter one.

In some embodiments of each of the methods, combinations, pharmaceutical compositions and uses of the present invention, the cancer is a solid tumor cancer. In some such embodiments, the cancer is a hematologic cancer. In some such
embodiments, the cancer is a cyclin E dominant cancer.

30 Examples of solid tumor cancers include, but are not limited to, small cell lung cancer, non-small cell lung cancer, squamous cell carcinoma, neuroendocrine prostate cancer (NEPC), breast cancer, brain cancer, head and neck cancer (including squamous cell carcinoma of the head and neck (SCCHN)), prostate cancer, ovarian cancer, bladder cancer, bone cancer, colorectal cancer, kidney cancer, liver cancer (including
35 hepatocellular carcinoma (HCC)), stomach cancer, pancreatic cancer, esophageal

cancer, cervical cancer, sarcoma, skin cancer, mesothelioma, malignant rhabdoid tumors, neuroblastoma, and diffuse intrinsic pontine glioma (DIPG).

Examples of hematologic cancers include, but are not limited to, acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL),
5 chronic myeloid leukemia (CML), small lymphocytic lymphoma, hairy cell leukemia, chronic myelomonocytic leukemia (CMML), adult T-cell leukemia/lymphoma (ATLL), Hodgkin's lymphoma, non-Hodgkin's lymphoma (NHL), multiple myeloma, plasmacytoma, plasma cell leukemia, and amyloidosis.

Examples of cyclin E dominant cancers include, but are not limited to, ovarian
10 cancer, breast cancer, prostate cancer, small cell lung cancer, colorectal cancer or pancreatic cancer.

The breast cancer is optionally node negative or node positive, estrogen receptor (ER) positive (ER+) or estrogen receptor (ER) negative (ER-), HER2 positive or HER2 negative, PR positive or PR negative, high grade or low grade, basal-like or luminal-like,
15 or a combination of these factors.

Breast cancers that have estrogen receptors are called ER-positive (or ER+) cancers. Breast cancers with progesterone receptors are called PR-positive (or PR+) cancers.

Triple-negative breast cancer cells don't have estrogen or progesterone receptors
20 and also don't make too much of the protein called HER2 (ER/PR/HER2 negative).

The retinoblastoma gene product, RB, is mutated or deleted in several tumor types, such as retinoblastoma, osteosarcoma and small-cell lung cancer, prostate cancer, uterine cancer, bladder cancer, liver cancer, ovarian cancer, esophageal cancer, stomach cancer, cervical cancer, glioblastoma, non-small cell lung cancer, lymphoma, breast
25 cancer and head and neck cancer. In some human cancers, the function of RB may be disrupted through neutralization by a binding protein, (e.g., Ishiji, T., The human papilloma virus-E7 protein in cervical carcinoma, *J Dermatol.* 2000 27: 73-86) or deregulation of pathways ultimately responsible for its phosphorylation.

By "RB pathway" it is meant the entire pathway of molecular signaling that includes
30 retinoblastoma protein (RB), and other protein/protein families in the pathway, including but not limited to CDK, E2f, atypical protein kinase C, and Skp2. Inactivation of the RB pathway often results from perturbation of p16INK4a, Cyclin D1, and CDK4.

As used herein, "RB" tumor suppressor gene refers to retinoblastoma tumor suppressor gene.

The term "RB+," "RB plus" or "RB positive" may be used to describe cells expressing detectable amounts of functional RB protein. RB positive includes wild-type and non-mutated RB protein. A wild-type RB (RB-WT) is generally understood to mean that form of the RB protein which is normally present in a corresponding population and which has the function which is currently assigned to this protein. RB positive may be cells which contain a functional RB gene. Cells which are RB positive may also be cells that can encode a detectable RB protein function.

The term "RB-," "RB minus," "RB deficient" or "RB negative" describe several types of cell where the function of RB is disrupted, including cells which produce no detectable amounts of functional RB protein. Cells that are RB negative may be cells which do not contain a functional RB gene. Cells that are RB negative may also be cells that can encode an RB protein, but in which the protein does not function properly.

In some embodiments of each of the methods, combinations and uses described herein, the cancer is characterized as retinoblastoma wild type (RB WT). In some embodiments of each of the methods, combinations and uses described herein, the cancer is characterized as RB positive. RB positive tumors contain at least some functional retinoblastoma genes.

In some embodiments of each of the methods, combinations, pharmaceutical compositions and uses described herein, the cancer is characterized as RB negative. RB negative cancers may be characterized by loss of function mutations, which may encode missense mutations (i.e., encode the wrong amino acid) or nonsense mutations (i.e., encode a stop codon). Alternatively, RB negative cancers may be characterized by deletion of all or part of the retinoblastoma gene.

As used herein, the term "patient" or "subject" refers to any subject for which therapy is desired or that is participating in a clinical trial, epidemiological study or used as a control, including humans and non-human animals, including veterinary subjects such as cattle, horses, dogs and cats. In a preferred embodiment, the subject is a human and may be referred to as a patient. Those skilled in the medical art are readily able to identify individual patients who are afflicted with cancer.

In some embodiments of each of the methods, combinations, pharmaceutical compositions and uses of the present invention, the combination or co-administration of two or more agents can be useful for treating individuals suffering from cancer who have primary or acquired resistance to ongoing therapies. The combination therapy provided herein may be useful for improving the efficacy and/or reducing the side effects of cancer therapies for individuals who do respond to such therapies.

As used herein, "drug resistance," "drug resistant cancer," "drug resistant cells," or "drug resistant disease" means a circumstance where a disease (e.g., cancer) does not respond to a therapeutic agent. Drug resistance can be intrinsic, which means that the disease has never been responsive to the therapeutic agent, or acquired, which means that the disease ceases responding to the agent or agents to which the disease had previously been responsive. For cancers, such therapeutic agent may be a chemotherapeutic drug such as colchicine, vinblastine, doxorubicin, *vinca* alkaloids, etoposide, taxanes, or other small molecules used in cancer chemotherapy (Aracytine and Daunorubicin in LAM therapy). Drug resistance may be associated with cancer and other conditions, such as bacterial, viral, protozoal, and fungal diseases.

As used herein, the term "combination therapy" refers to the administration of each agent of the combination therapy of the present invention, either alone or in a medicament, either simultaneously, separately or sequentially, as mixed or individual dosages.

As used herein, the term "simultaneously," "simultaneous administration," "administered simultaneously," "concurrently," or "concurrent administration" means that the agents are administered at the same point in time or immediately following one another, but that the agents can be administered in any order. For example, in the latter case, the two or more agents are administered at times sufficiently close that the results observed are indistinguishable from those achieved when the agents are administered at the same point in time. The term simultaneous includes the administration of each agent of the combination therapy of the present invention in the same medicament.

The agents of the present invention can be administered completely separately or in the form of one or more separate compositions. For example, the agents may be given separately at different times during the course of therapy (in a chronologically staggered manner, especially a sequence-specific manner) in such time intervals that the combination therapy is effective in treating cancer.

As used herein, the term "sequential," "sequentially," "administered sequentially," or "sequential administration" refers to the administration of each agent of the combination therapy of the present invention, either alone or in a medicament, one after the other, wherein each agent can be administered in any order. Sequential administration may be particularly useful when the therapeutic agents in the combination therapy are in different dosage forms, for example, one agent is a tablet and another agent is a sterile liquid, and/or the agents are administered according to different dosing schedules, for example,

one agent is administered daily, and the second agent is administered less frequently such as weekly.

As used herein, "in combination with," "in conjunction with" or "combined administration" refers to administration of one agent in addition to at least one other agent.

5 As such, "in combination with," "in conjunction with" or "combined administration" refers to administration of one agent before, during, or after administration of at least one other agent to the individual. The administration of two or more agents are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

10 A "combination" or "pharmaceutical combination" refers to a combination of any two or more agents as described herein, e.g., any CDK2 inhibitor described herein with any CDC25A inhibitor described herein. These two or more agents may (but do not necessarily) belong to different classes of agents.

In some embodiments of each of the methods, combinations and uses described
15 herein, the combination therapy is administered to a subject in a single dose. In some embodiments of each of the methods, combinations and uses described herein, the combination therapy is administered to a subject in multiple doses. In some embodiments of each of the methods, combinations and uses described herein, an amount of a combination is administered to a subject periodically at regular intervals (e.g., 1, 2, 3, 4,
20 5, 6, 7, 8, 9, 10 or more times every 1, 2, 3, 4, 5, or 6 days, or every 1, 2, 3, 4, 5, 6, 7, 8, or 9 weeks, or every 1, 2, 3, 4, 5, 6, 7, 8, 9 months or longer).

In some embodiments of each of the methods, combinations and uses described herein, the combination therapy is administered to a subject at a predetermined interval (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more times every 1, 2, 3, 4, 5, or 6 days, or every 1, 2,
25 3, 4, 5, 6, 7, 8, or 9 weeks, or every 1, 2, 3, 4, 5, 6, 7, 8, 9 months or longer).

In some embodiments of each of the methods, combinations and uses described herein, the present invention relates to combinations of two or more agents for simultaneous, separate or sequential administration, in particular for the treatment or prevention of cancer. For example, the individual agents of the combination of the
30 present invention can be administered separately at different times in any order during the course of therapy or concurrently in divided or single combination forms.

The terms "concurrent administration," "administration in combination," "simultaneous administration" or "administered simultaneously," as used herein, means that the agents are administered at the same point in time or immediately following one
35 another. For example, in the latter case, the two agents are administered at times

sufficiently close that the results observed are indistinguishable from those achieved when the agents are administered at the same point in time.

In some embodiments of each of the methods, combinations and uses described herein, the agents of the present invention can be administered completely separately or
5 in the form of one or more separate compositions. For example, the agents may be given separately at different times during the course of therapy (in a chronologically staggered manner, especially a sequence-specific manner) in such time intervals that the combination therapy is effective in treating cancer.

As used herein, the term “sequentially” refers to a treatment in which administration
10 of a first treatment, such as administration of first agent, follows administration of a second treatment, such as administration of a second agent.

In some embodiments of each of the methods, combinations and uses described herein, the dosage of the individual agents of the combination may require more frequent administration of one of the agent(s) as compared to the other agent(s) in the
15 combination. Therefore, to permit appropriate dosing, packaged pharmaceutical products may contain one or more dosage forms that contain the combination of agents, and one or more dosage forms that contain one of the combination of agents, but not the other agent(s) of the combination.

As used herein, the term “single formulation” refers to a single carrier or vehicle
20 formulated to deliver effective amounts of both therapeutic agents to a subject. The single vehicle is designed to deliver an effective amount of each of the agents, along with any pharmaceutically acceptable carriers or excipients. In some embodiments, the vehicle is a tablet, capsule, pill, or a patch. In other embodiments, the vehicle is a solution or a suspension.

The term “unit dose,” is used herein to mean simultaneous administration of both
25 agents together, in one dosage form, to the subject being treated. In some embodiments, the unit dose is a single formulation. In certain embodiments, the unit dose includes one or more vehicles such that each vehicle includes an effective amount of at least one of the agents along with pharmaceutically acceptable carriers and excipients. In some
30 embodiments, the unit dose is one or more tablets, powder, capsules, pills, patches, sustained release formulations, solution suspension, for parenteral injection as a sterile solution, suspension or emulsion, for topical administration as an ointment or cream or for rectal administration as a suppository. Exemplary parenteral administration forms include solutions or suspensions of active compounds in sterile aqueous solutions, for

example, aqueous propylene glycol or dextrose solutions. Such dosage forms may be suitably buffered, if desired.

As used herein, the term “advanced” as it relates to a cancer, includes locally advanced (non-metastatic) disease and metastatic disease.

As used herein, the term “treat” or “treating” a cancer means to administer a combination therapy according to the present invention to a subject having cancer, or diagnosed with cancer, to achieve at least one positive therapeutic effect, such as, for example, reduced number of cancer cells, reduced tumor size, reduced rate of cancer cell infiltration into peripheral organize, or reduced rate of tumor metastases or tumor growth, reversing, stopping, controlling, slowing, interrupting, arresting, alleviating, and/or inhibiting the progression or severity of a sign, symptom, disorder, condition, or disease, but does not necessarily involve a total elimination of all disease-related signs, symptoms, conditions, or disorders. The term “treatment,” as used herein, unless otherwise indicated, refers to the act of treating as “treating” is defined immediately above. The term “treating” also includes adjuvant and neo-adjuvant treatment of a subject. For the purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: reducing the proliferation of (or destroying) neoplastic or cancerous cell; inhibiting metastasis or neoplastic cells; shrinking or decreasing the size of tumor; remission of the cancer; decreasing symptoms resulting from the cancer; increasing the quality of life of those suffering from the cancer; decreasing the dose of other medications required to treat the cancer; delaying the progression the cancer; curing the cancer; overcoming one or more resistance mechanisms of the cancer; and / or prolonging survival of patients the cancer. Positive therapeutic effects in cancer can be measured in a number of ways (see, for example, W. A. Weber, Assessing Tumor Response to Therapy, *J. Nucl. Med.* 2009, 50:1S-10S). For example, with respect to tumor growth inhibition (T/C), according to the National Cancer Institute (NCI) standards, a T/C less than or equal to 42% is the minimum level of anti-tumor activity. A T/C <10% is considered a high anti-tumor activity level, with $T/C (\%) = \frac{\text{median tumor volume of the treated}}{\text{median tumor volume of the control}} \times 100$.

In some embodiments, the treatment achieved by a combination of the present invention is any of the complete response (CR), disease free survival (DFS), duration of response (DoR), overall response (OR), overall response rate (ORR), overall survival (OS), progressive disease (PD), progression free survival (PFS), partial response (PR) and stable disease (SD).

As used herein, the term "complete response" or "CR" means the disappearance of all signs of cancer (e.g., disappearance of all target lesions) in response to treatment. This does not always mean the cancer has been cured.

5 As used herein, the term "disease-free survival" (DFS) means the length of time after primary treatment for a cancer ends that the patient survives without any signs or symptoms of that cancer.

As used herein, the term "duration of response" (DoR) means the length of time that a tumor continues to respond to treatment without the cancer growing or spreading. Treatments that demonstrate improved DoR can produce a durable, meaningful delay in disease progression.

10

As used herein, the terms "objective response" and "overall response" refer to a measurable response, including complete response (CR) or partial response (PR). The term "overall response rate" (ORR) refers to the sum of the complete response (CR) rate and the partial response (PR) rate.

15 As used herein, the term "overall survival" (OS) means the length of time from either the date of diagnosis or the start of treatment for a disease, such as cancer, that patients diagnosed with the disease are still alive. OS is typically measured as the prolongation in life expectancy in patients who receive a certain treatment as compared to patients in a control group (*i.e.*, taking either another drug or a placebo).

20 As used herein, the term "progressive disease" or "PD" refers to a cancer that is growing, spreading or getting worse. In some embodiments, PR refers to at least a 20% increase in the SLD of target lesions, taking as reference the smallest SLD recorded since the treatment started, or to the presence of one or more new lesions.

As used herein, the term "progression free survival" or "PFS" refers to the length of time during and after treatment during which the disease being treated (e.g., cancer) does not get worse. PFS, also referred to as "Time to Tumor Progression", may include the amount of time patients have experienced a CR or PR, as well as the amount of time patients have experienced SD.

25

As used herein, the term "partial response" or "PR" refers to a decrease in the size of one or more tumors or lesions, or in the extent of cancer in the body, in response to treatment. For example, in some embodiments, PR refers to at least a 30% decrease in the sum of the longest diameters (SLD) of target lesions, taking as reference the baseline SLD.

30

As used herein, the term "stable disease" (SD) refers to a cancer that is neither decreasing nor increasing in extent or severity.

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As used herein, the term "sustained response" refers to the sustained effect on reducing tumor growth after cessation of a treatment. For example, the tumor size may be the same size or smaller as compared to the size at the beginning of the medicament administration phase. In some embodiments, the sustained response has a duration of at least the same as the treatment duration, at least 1.5x, 2x, 2.5x, or 3x length of the treatment duration, or longer.

The anti-cancer effect of the method of treating cancer, including "objective response," "complete response," "partial response," "progressive disease," "stable disease," "progression free survival," "duration of response," as used herein, may be defined and assessed by the investigators using RECIST v1.1 (Eisenhauer *et al.*, New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1), *Eur J of Cancer*, 2009; 45(2):228-47). In some embodiments of each of the methods, combinations and uses herein, the therapeutic effect achieved by the compound of Formula (I), e.g., CDK2 inhibitor in combination with a CDC25A inhibitor and/or an additional anti-cancer agent as further described herein, is defined by reference to any of the following: complete response (CR), disease free survival (DFS), duration of response (DoR), overall response rate (ORR), overall survival (OS), partial response (PR), or progression free survival (PFS). In some embodiments, response to a combination of the invention is any of PR, CR, PFS, DFS, OR or OS that is assessed using Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 response criteria.

The treatment regimen for a method, combination or use of the invention that is effective to treat cancer in a subject may vary according to factors such as the disease state, age, and weight of the patient, and the ability of the therapy to elicit an anti-cancer response in the subject. While an embodiment of any of the aspects of the present invention may not be effective in achieving a positive therapeutic effect in every subject, it should do so in a statistically significant number of subjects as determined by any statistical test known in the art such as the Student's t-test, the chi²-test the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstrat-testy and the Wilcon on-test.

The term "treatment" also encompasses *in vitro* and *ex vivo* treatment, e.g., of a cell, by a reagent, diagnostic, binding compound, or by another cell.

As used herein, the term "diagnosis" refers to the identification or classification of a molecular or pathological state, disease or condition (e.g., cancer). For example, "diagnosis" may refer to identification of a particular type of cancer. "Diagnosis" may also refer to the classification of a particular subtype of cancer, e.g., by histopathological

criteria, or by molecular features (e.g., a subtype characterized by expression of one or a combination of biomarkers (e.g., particular genes or proteins encoded by said genes)).

As used herein, the term "aiding diagnosis" refers to methods that assist in making a clinical determination regarding the presence, or nature, of a particular type of symptom or condition of a disease or disorder (e.g., cancer). For example, a method of aiding diagnosis of a disease or condition (e.g., cancer) can comprise measuring certain biomarkers in a biological sample from an individual.

As used herein, the term "sample" refers to a composition that is obtained or derived from a subject and/or individual of interest that contains a cellular and/or other molecular entity that is to be characterized and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics. For example, the phrase "disease sample" and variations thereof refers to any sample obtained from a subject of interest that would be expected or is known to contain the cellular and/or molecular entity that is to be characterized. Samples include, but are not limited to, primary or cultured cells or cell lines, cell supernatants, cell lysates, platelets, serum, plasma, vitreous fluid, lymph fluid, synovial fluid, follicular fluid, seminal fluid, amniotic fluid, milk, whole blood, blood-derived cells, urine, cerebro-spinal fluid, saliva, sputum, tears, perspiration, mucus, tumor lysates, and tissue culture medium, tissue extracts such as homogenized tissue, tumor tissue, cellular extracts, and combinations thereof.

By "tissue sample" or "cell sample" is meant a collection of similar cells obtained from a tissue of a subject or individual. The source of the tissue or cell sample may be solid tissue as from a fresh, frozen and/or preserved organ, tissue sample, biopsy, and/or aspirate; blood or any blood constituents such as plasma; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from any time in gestation or development of the subject. The tissue sample may also be primary or cultured cells or cell lines. Optionally, the tissue or cell sample is obtained from a disease tissue/organ. The tissue sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like.

A "reference sample," "reference cell," "reference tissue," "control sample," "control cell," or "control tissue," as used herein, refers to a sample, cell, tissue, standard, or level that is used for comparison purposes. In one embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from a healthy and/or non-diseased part of the body (e.g., tissue or cells) of the same subject or individual. For example, healthy and/or non-diseased cells or tissue adjacent to the

diseased cells or tissue (e.g., cells or tissue adjacent to a tumor). In another embodiment, a reference sample is obtained from an untreated tissue and/or cell of the body of the same subject or individual. In yet another embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from a healthy and/or non-diseased part of the body (e.g., tissues or cells) of an individual who is not the subject or individual. In even another embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from an untreated tissue and/or cell of the body of an individual who is not the subject or individual.

As used herein, the term "pharmaceutical composition" or "pharmaceutical composition of the present invention" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile. In some embodiments, a pharmaceutical composition of the present invention refers to a CDK2 inhibitor, CDC25A inhibitor, a mixture of one or more of the CDK2 inhibitors, and/or a mixture of one or more of the CDC25A inhibitors described herein, or a pharmaceutically acceptable salt, solvate, hydrate or prodrug thereof as an active ingredient, and at least one pharmaceutically acceptable carrier or excipient. In some embodiments, the pharmaceutical composition comprises two or more pharmaceutically acceptable carriers and/or excipients. In other embodiments, the pharmaceutical composition further comprises at least one additional anti-cancer therapeutic agent.

In some embodiments, a pharmaceutical composition of the present invention further comprises at least one additional anti-cancer therapeutic agent or a palliative agent. In some such embodiments, the at least one additional agent is an anti-cancer therapeutic agent as described below. In some such embodiments, the combination provides an additive, greater than additive, or synergistic anti-cancer effect.

As used herein, a "pharmaceutically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the active compound or therapeutic agent.

The pharmaceutical acceptable carrier may comprise any conventional pharmaceutical carrier or excipient. The choice of carrier and/or excipient will to a large extent depend on factors such as the particular mode of administration, the effect of the excipient on solubility and stability, and the nature of the dosage form. Suitable pharmaceutical carriers include inert diluents or fillers, water and various organic solvents

(such as hydrates and solvates). The pharmaceutical compositions may, if desired, contain additional ingredients such as flavorings, binders, excipients and the like. Thus, for oral administration, tablets containing various excipients, such as citric acid may be employed together with various disintegrants such as starch, alginic acid and certain complex silicates and with binding agents such as sucrose, gelatin and acacia. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often useful for tableting purposes. Solid compositions of a similar type may also be employed in soft and hard filled gelatin capsules. Non-limiting examples of materials, therefore, include lactose or milk sugar and high molecular weight polyethylene glycols. When aqueous suspensions or elixirs are desired for oral administration the active compound therein may be combined with various sweetening or flavoring agents, coloring matters or dyes and, if desired, emulsifying agents or suspending agents, together with diluents such as water, ethanol, propylene glycol, glycerin, or combinations thereof.

The pharmaceutical composition may, for example, be in a form suitable for oral administration as a tablet, capsule, pill, powder, sustained release formulation, solution or suspension, for parenteral injection as a sterile solution, suspension or emulsion, for topical administration as an ointment or cream, or for rectal administration as a suppository.

Exemplary parenteral administration forms include solutions or suspensions of an active compound in a sterile aqueous solution, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms may be suitably buffered, if desired.

The pharmaceutical composition may be in unit dosage forms suitable for single administration of precise amounts.

Pharmaceutical compositions suitable for the delivery of the therapeutic agents of the combination therapies of the present invention, and methods for their preparation will be readily apparent to those skilled in the art. Such compositions and methods for their preparation may be found, for example, in 'Remington's Pharmaceutical Sciences', 19th Edition (Mack Publishing Company, 1995), the disclosure of which is incorporated herein by reference in its entirety.

An "effective amount" is at least the minimum amount required to affect a measurable improvement or prevention of a particular disorder. An effective amount herein may vary according to factors such as the disease state, age, sex, and weight of

the patient, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the treatment are outweighed by the therapeutically beneficial effects. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In the case of cancer or tumor, an effective amount of the drug may have the effect in reducing the number of cancer cells; reducing the tumor size; inhibiting (*i.e.*, slow to some extent or desirably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and desirably stop) tumor metastasis; inhibiting to some extent tumor growth; and/or relieving to some extent one or more of the symptoms associated with the disorder. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

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

As used herein, the term "ameliorating" with reference to a disease, disorder or condition, refers to any observable beneficial effect of the treatment. Treatment need not be absolute to be beneficial to the subject. For example, ameliorating means a lessening or improvement of one or more symptoms of a disease, disorder or condition as compared to not administering a therapeutic agent of a method or regimen of the present invention. Ameliorating also includes shortening or reduction in duration of a symptom.

As used herein, an “effective dosage” or “effective amount” of a drug, compound or pharmaceutical composition is an amount sufficient to affect any one or more beneficial or desired, including biochemical, histological and / or behavioral symptoms, of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, a “therapeutically effective amount” refers to that amount of a compound being administered which will relieve to some extent one or more of the symptoms of the disorder being treated. In reference to the treatment of cancer, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of the tumor, (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis, (3) inhibiting to some extent (that is, slowing to some extent, preferably stopping) tumor growth or tumor invasiveness, (4) relieving to some extent (or, preferably, eliminating) one or more signs or symptoms associated with the cancer, (5) decreasing the dose of other medications required to treat the disease, and / or (6) enhancing the effect of another medication, and / or delaying the progression of the disease of patients.

An effective dosage can be administered in one or more administrations. For the purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of drug, compound or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound or pharmaceutical composition.

As used herein, a “sub-therapeutic amount” of drug, compound or pharmaceutical composition is an amount less than the effective amount for that drug, compound or pharmaceutical composition, but when combined with an effective or sub-therapeutic amount of another drug, compound or pharmaceutical composition can produce a result desired by the physician, due to, for example, synergy in the resulting efficacious effects, or reduced side effects.

The term “bioequivalent” refers to the United States Food and Drug Administration (FDA) guidelines for pharmaceutical formulations. For example, according to the FDA the term bioequivalence is defined as “the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study” (United States Food and Drug Administration, “Guidance for Industry:

Bioavailability and Bioequivalence Studies for Orally Administered Drug Products-General Considerations," 2003, Center for Drug Evaluation and Research).

"Tumor" as it applies to a subject diagnosed with, or suspected of having, a cancer refers to a malignant or potentially malignant neoplasm or tissue mass of any size and includes primary tumors and secondary neoplasms. A solid tumor is an abnormal growth or mass of tissue that usually does not contain cysts or liquid areas. Examples of solid tumors are sarcomas, carcinomas, and lymphomas. Leukemia's (cancers of the blood) generally do not form solid tumors (National Cancer Institute, Dictionary of Cancer Terms).

"Tumor burden" also referred to as a "tumor load", refers to the total amount of tumor material distributed throughout the body. Tumor burden refers to the total number of cancer cells or the total size of tumor(s), throughout the body, including lymph nodes and bone marrow. Tumor burden can be determined by a variety of methods known in the art, such as, e.g., using calipers, or while in the body using imaging techniques, e.g., ultrasound, bone scan, computed tomography (CT), or magnetic resonance imaging (MRI) scans.

As used herein, the term "tumor size" refers to the total size of the tumor which can be measured as the length and width of a tumor. Tumor size may be determined by a variety of methods known in the art, such as, e.g., by measuring the dimensions of tumor(s) upon removal from the subject, e.g., using calipers, or while in the body using imaging techniques, e.g., bone scan, ultrasound, CR or MRI scans.

The term "additive" is used to mean that the result of the combination of two or more agents is no greater than the sum of each agent individually. In one embodiment, the combination of agents described herein displays a synergistic effect. The term "synergy" or "synergistic" are used to mean that the result of the combination of two or more agents is greater than the sum of each agent individually. This improvement in the disease, condition or disorder being treated is a "synergistic" effect. A "synergistic amount" is an amount of the combination of the two or more agents that results in a synergistic effect, as "synergistic" is defined herein. A "synergistic combination" refers to a combination of agents which produces a synergistic effect *in vivo*, or alternatively *in vitro* as measured according to the methods described herein.

Determining a synergistic interaction between two or more agents, the optimum range for the effect and absolute dose ranges of each agent for the effect may be definitively measured by administration of the agents over different dose ranges, and/or dose ratios to subjects in need of treatment. However, the observation of synergy in *in*

vitro models or *in vivo* models can be predictive of the effect in humans and other species and *in vitro* models or *in vivo* models exist, as described herein, to measure a synergistic effect. The results of such studies can also be used to predict effective dose and plasma concentration ratio ranges and the absolute doses and plasma concentrations required in humans and other species such as by the application of pharmacokinetic and / or pharmacodynamics methods.

A “nonstandard clinical dosing regimen,” as used herein, refers to a regimen for administering a substance, agent, compound or composition, which is different to the amount, dose or schedule typically used for that substance, agent, compound or composition in a clinical setting. A “non-standard clinical dosing regimen,” includes a “non-standard clinical dose” or a “nonstandard dosing schedule”.

A “low dose amount regimen,” as used herein refers to a dosing regimen where one or more of the substances, agents, compounds or compositions in the regimen are dosed at a lower amount or dose than typically used in a clinical setting for that agent, for example when that agent is dosed as a singleton therapy.

As used herein, the term “pharmaceutically acceptable salt” refers to those salts which retain the biological effectiveness and properties of the parent compound. The phrase “pharmaceutically acceptable salt(s)”, as used herein, unless otherwise indicated, includes salts of acidic or basic groups which may be present in the compounds of the formulae disclosed herein. For example, the compounds of the invention that are basic in nature may be capable of forming a wide variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds of those that form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions. Examples of anions suitable for mono- and di- acid addition salts include, but are not limited to, acetate, asparatate, benzenesulfonate, benzoate, besylate, bicarbonate, bisulfate, bitartrate, bromide, calcium edetate, camsylate, carbonate, chloride, citrate, decanoate, edetate, edislyate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollate, hexanoate, hexylresorcinate, hydrabamine, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, octanoate, oleate, pamoate (embonate), pantothenate, phosphate, polygalacturonate, propionate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, teoclate, tosylate, triethiodode, and valerate salts. Alternatively, compounds that are acidic in nature may be capable of forming base salts with various pharmacologically acceptable cations which form non-toxic base salts. Such non-toxic

base salts include, but are not limited to, those derived from such pharmacologically acceptable cations such as alkali metal cations (e.g., potassium and sodium) and alkaline earth metal cations (e.g., calcium and magnesium), ammonium or water-soluble amine addition salts such as N-methylglucamine-(meglumine), and the lower alkanolammonium and other base salts of pharmaceutically acceptable organic amines. Examples of cations suitable for such salts include alkali metal or alkaline-earth metal salts and other cations, including aluminium, arginine, benzathine, calcium, chlorprocaine, choline, diethanolamine, ethanolamine, ethylenediamine, lysine, magnesium, histidine, lithium, meglumine, potassium, procaine, sodium, triethylamine and zinc. Salts may be prepared by conventional techniques. Hemisalts of acids and bases may also be formed, for example, hemisulphate and hemicalcium salts. For a review on suitable salts, see Handbook of Pharmaceutical Salts: Properties, Selection, and Use by Stahl and Wermuth (Wiley-VCH, 2002). Methods for making pharmaceutically acceptable salts are known to those of skill in the art.

As used herein, the term "solvate" describes a molecular complex comprising a compound described herein and one or more pharmaceutically acceptable solvent molecules, for example, water and ethanol.

A "package insert" refers to instructions customarily included in commercial packages of medicaments that contain information about the indications customarily included in commercial packages of medicaments that contain information about the indications, usage, dosage, administration, contraindications, other medicaments to be combined with the packaged product, and/or warnings concerning the use of such medicaments, etc.

The compounds described herein may also exist in unsolvated and solvated forms. Accordingly, some embodiments relate to the hydrates and solvates of the compounds described herein.

Compounds described herein containing one or more asymmetric carbon atoms can exist as two or more stereoisomers. Where a compound described herein contains an alkenyl or alkenylene group, geometric cis/trans (or Z/E) isomers are possible. Where structural isomers are interconvertible *via* a low energy barrier, tautomeric isomerism ('tautomerism') can occur. This can take the form of proton tautomerism in compounds described herein containing, for example, an imino, keto, or oxime group, or so-called valence tautomerism in compounds which contain an aromatic moiety. A single compound may exhibit more than one type of isomerism.

The compounds of the embodiments described herein include all stereoisomers (e.g., *cis* and *trans* isomers) and all optical isomers of compounds described herein (e.g., *R* and *S* enantiomers), as well as racemic, diastereomeric and other mixtures of such isomers. While all stereoisomers are encompassed within the scope of our claims, one skilled in the art will recognize that particular stereoisomers may be preferred.

In some embodiments, the compounds described herein can exist in several tautomeric forms, including the enol and imine form, and the keto and enamine form and geometric isomers and mixtures thereof. All such tautomeric forms are included within the scope of the present embodiments. Tautomers exist as mixtures of a tautomeric set in solution. In solid form, usually one tautomer predominates. Even though one tautomer may be described, the present embodiments include all tautomers of the present compounds.

Included within the scope of the present embodiments are all stereoisomers, geometric isomers and tautomeric forms of the compounds described herein, including compounds exhibiting more than one type of isomerism, and mixtures of one or more thereof. Also included are acid addition or base salts wherein the counterion is optically active, for example, d-lactate or l-lysine, or racemic, for example, dl-tartrate or dl-arginine.

The present embodiments also include atropisomers of the compounds described herein. Atropisomers refer to compounds that can be separated into rotationally restricted isomers.

Cis/trans isomers may be separated by conventional techniques well known to those skilled in the art, for example, chromatography and fractional crystallization.

Conventional techniques for the preparation/isolation of individual enantiomers include chiral synthesis from a suitable optically pure precursor or resolution of the racemate (or the racemate of a salt or derivative) using, for example, chiral high-pressure liquid chromatography (HPLC).

Alternatively, the racemate (or a racemic precursor) may be reacted with a suitable optically active compound, for example, an alcohol, or, in the case where a compound described herein contains an acidic or basic moiety, a base or acid such as 1-phenylethylamine or tartaric acid. The resulting diastereomeric mixture may be separated by chromatography and/or fractional crystallization and one or both of the diastereoisomers converted to the corresponding pure enantiomer(s) by means well known to a skilled person.

METHODS, COMBINATIONS, USES AND MEDICAMENTS

The present invention provides methods, combinations, uses and medicaments that may be useful for treating cancer. Some embodiments provided herein result in one or more of the following effects: (1) inhibiting cancer cell proliferation; (2) inhibiting cancer cell invasiveness; (3) inducing apoptosis of cancer cells; (4) inhibiting cancer cell metastasis; (5) inhibiting angiogenesis; and/or (6) overcoming one or more resistance mechanisms relating to a cancer treatment.

Exemplary methods and materials are described herein, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. The materials, methods, and examples are illustrative only and not intended to be limiting.

General Methods

The practice of the present invention employs, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA, which are within the skill of the art. See Sambrook, Fritsch and Maniatis, *Molecular Cloning, A Laboratory Manual*; 1982 & 1989 2nd Edition, 2001 3rd Edition.; Wu, *Recombinant DNA, Methods in enzymology* 1993, Vol. 217, p754.

Standard methods also appear in Ausbel, *et al.*, *Current Protocols in Molecular Biology*, 2001 Vols.1-4, which describes cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4).

Several aspects of the present invention relate to vector systems comprising one or more vectors, or vectors as such. Vectors can be designed for expression of CRISPR transcripts (e.g. nucleic acid transcripts, proteins, or enzymes) in prokaryotic or eukaryotic cells. For example, CRISPR transcripts can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Keown, *et al.*, *Methods for introducing DNA into mammalian cells, in Methods in Enzymology 185: Gene Expression Technology* 1990 pp. 527–537. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Vectors may be introduced and propagated in a prokaryote. In some embodiments, a prokaryote is used to amplify copies of a vector to be introduced into a

eukaryotic cell or as an intermediate vector in the production of a vector to be introduced into a eukaryotic cell (e.g., amplifying a plasmid as part of a viral vector packaging system). In some embodiments, a prokaryote is used to amplify copies of a vector and express one or more nucleic acids, such as to provide a source of one or more proteins for delivery to a host cell or host organism.

The quantification of cells can be performed by known detection methods such as fluorometrically or enzymatically.

The cells can be quantitated either enzymatically, for example, by measuring lactate dehydrogenase (LDH) activity as described in Neurath, A.R. *et al.*, Bovine 3-Lactoglobulin Modified by 3-Hydroxyphthalic Anhydride Blocks the CD4 Cell Receptor for HIV, *Nature Medicine* 1996, 2, 230-234 or fluorometrically, for example, by using the ("CyQUANT"™ Assay Kit (Molecular Probes, Inc., Eugene, OR), with similar results. For example, the cells can be quantitated fluorometrically by using the CyQUANT™ Cell Proliferation Assay Kit, which provides a rapid and sensitive procedure for determining the density of cells in culture. The assay has a linear detection range extending from 50 or fewer to at least 50,000 cells in 200 µL volumes and thus can be used for cell proliferation studies, as well as for routine cell counts. The CyQUANT™ assay can detect much lower cell numbers than Neutral Red or methylene blue assays. Unlike procedures that rely on the conversion of tetrazolium dyes to blue formazan products or on ³H thymidine incorporation assays, the CyQUANT™ method is rapid and does not rely on cellular metabolic activity. The CyQUANT® Direct assay is a fluorescence-based proliferation and cytotoxicity assay for microplate readers with a linear detection range from less than 100 to 20,000 cells per well in most cell types. The no-wash, homogenous format and fast add-mix-read protocol makes the CyQUANT® Direct assay ideal for HTS applications. The assay can be completed in one hour, with no washes, no cell lysis, or temperature equilibrations required. The signal is stable for several hours, affording additional workflow convenience.

Methods of Identifying Cancer Treatment Targets

Because of its high efficiency and accuracy, the CRISPR-Cas9 functional genomic screening has recently emerged as a potentially powerful tool in drug target discovery. Among its many applications, CRISPR-Cas9 has shown an unprecedented clinical potential to discover novel targets for cancer therapy and to dissect chemical-genetic interactions, providing insight into how tumors respond to drug treatment.

CRISPR/Cas9 screens are platforms for oncology target discovery because they can uncover unique dependencies of oncogene addiction, lineage-specific regulators, synthetic lethal vulnerabilities of drug treatment, vulnerabilities of tumor immune evasion in genetically engineered mouse models, and physiologically relevant targets of the tumor microenvironment *in vivo* (Wang, *et al.*, Genetic screens in human cells using the CRISPR-Cas9 system, *Science*, 2014, 343, 80-84; Tsherniak, *et al.*, Defining a Cancer Dependency Map, *Cell*, 2017, 170, 564-576 e516; Chen, *et al.*, Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis, *Cell*, 2015, 160, 1246-1260; Manguso, *et al.*, In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target, *Nature*, 2017, 547, 413-418; Konermann, *et al.* Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex, *Nature*, 2015, 517, 583-588).

The present invention further provides a method for identifying cancer treatment targets. In some cases, the method comprises the steps of: (a) generating normal and cancer cells harboring a CRISPR/Cas effector polypeptide; (b) inhibiting expression of a target gene in the normal and cancer cells generated in step (a) by stably introducing an sgRNA expression construct directed to the target gene, thereby inhibiting expression of the target gene; (c) monitoring one or more molecular features and/or phenotypes (e.g., disease phenotypes) in the cells following inhibition of target gene expression; and/or (d) designating the molecular feature and disease phenotype as a target gene knockdown-related features and phenotypes, if improvement in the molecular feature or phenotype is observed following step (b). In some embodiments, a method for identifying a cancer treatment target, the method comprises: (a) introducing a CRISPR/Cas effector polypeptide into a cancer cell and into a corresponding normal cell of the same cell type as the cancer cell; (b) introducing a CRISPR/Cas guide RNA targeting a gene of interest into the normal and cancer cells generated in step (a), thereby reducing expression of the target gene; (c) monitoring one or more molecular features and/or phenotypes in the cells following reduction of target gene expression; and (d) where the one or more molecular feature and disease phenotype indicates a reduction in the cancerous state of the cancer cell, identifying the target gene as a candidate cancer treatment target. In some cases, a method for identifying a cancer treatment target, the method comprises: a) introducing into a cancer cell and into a corresponding normal cell of the same cell type as the cancer cell a CRISPR/Cas system comprising: i) a CRISPR/Cas effector polypeptide; and ii) one or more CRISPR/Cas guide RNAs, wherein said introducing reduces expression of a target gene targeted by the guide RNA (e.g., a target gene to which the guide RNA hybridizes); and b) assessing the effect of the reduced expression of the target gene on

one or more molecular features and/or phenotypes in the cells. Where assessment of the one or more molecular features and/or phenotypes in the cells indicates that reduced expression of the target gene reduces the cancer phenotype of the cancer cell, the target gene is identified as a candidate target gene for cancer treatment.

5 The present disclosure also provides certain compositions and methods for delivering CRISPR/CRISPR-associated (Cas) 9-based system and multiple gRNAs to target one or more endogenous genes. Co-transfection of multiple sgRNAs targeted to a single promoter allow for synergistic activation, however, co-transfection of multiple plasmids leads to variable expression levels in each cell due to differences in copy
10 number. Additionally, gene activation following transfection is transient due to dilution of plasmid DNA over time. Moreover, many cell types are not easily transfected, and transient gene expression may not be sufficient for inducing a therapeutic effect. To address these limitations, a single lentiviral system may be developed to express Cas9 and sgRNAs from independent promoters.

15 In one embodiment, the present invention relates to chemical-genetic methods that are based on systematically profiling the effects of genetic perturbations on drug sensitivity. In some such embodiments, application of these methods to mammalian systems has been facilitated by the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR associated (Cas) 9 (CRISPR-Cas9)-based approaches, to
20 repress, induce, or delete a given gene and determine the resulting effects on drug sensitivity.

In one embodiment, sgRNA protospacer sequences were selected for every human and mouse gene in the genome. In some embodiments, sgRNA expression vectors were constructed by assembling DNA sequences and flanking guide
25 protospacers. Methods for individual sgRNA library design are well known in the art (e.g., Hart, Traver, *et al.*, Evaluation and design of genome-wide CRISPR/SpCas9 knockout screens, *G3: Genes, Genomes, Genetics* 7.8, 2017, 2719-2727 and Tarumoto, Y. *et al.*, LKB1, salt-inducible kinases, and MEF2C are linked dependencies in acute myeloid leukemia *Molecular cell* 2018, 69.6: 1017-1027).

30 Furthermore, the lentiviral platform provides the potent and sustained levels of gene expression that will facilitate therapeutic applications of the CRISPR/Cas9 system in primary cells. Finally, this system may be used for editing multiple genes simultaneously, such as the concurrent knockout of several oncogenes. Cell lines stably expressing Cas9 were generated by lentiviral transduction. In one embodiment, cells with
35 stable Cas9 expression were infected in three biological replicates per cell line with

lentiviral sgRNA pools. In some such embodiments, each cell line was simultaneously screened with different CDK inhibitors. In specific embodiments, Crispr screening were carried out to identify synthetic lethal hit for the CDK2 Inhibition.

In a particular embodiment of each of the foregoing, multiple CRISPR screens identified CDC25A as a strong synthetic lethal hit for both CDK2-selective and CDK2/4/6 inhibition in small cell lung, pancreatic and ER+ breast cancer models. In further embodiments of each of the foregoing, CDC25A was validated as a synthetic lethal target with CDK2 inhibition by CRISPR knockout in small cell lung cancer (SCLC) cell models. In additional embodiments, CDC25A was validated as a synthetic lethal target with CDK2 inhibition by shRNA knockdown in small cell lung cancer (SCLC) cell model H82 cells.

Methods for determining the capacity of a compound to be CDC25A phosphatase inhibitor are well known to the person skilled in the art. In a preferred embodiment, the inhibitor specifically binds to CDC25 in a sufficient manner to inhibit the phosphatase activity of CDC25A. Binding to CDC25A and inhibition of the phosphatase activity of CDC25A may be determined by any competing assays well known in the art. For example, the assay may consist in determining the ability of the agent to be tested as CDC25A phosphatase inhibitor to bind to CDC25A. The binding ability is reflected by the K_d measurement.

In specific embodiments, an inhibitor that "specifically binds to CDC25A" is intended to refer to an inhibitor that binds to human CDC25A polypeptide with a K_D of 1 μM or less, 100 nM or less, 10 nM or less, or 3 nM or less. A competitive assay may be performed to determine the ability of the agent to inhibit phosphatase activity of CDC25A. The functional assays may be envisaged such evaluating the phosphorylation of the CDC25A substrate (*i.e.*, CDK1 or CDK2). Such functional tests are described in Brezak *et. al.*, PM-20, a novel inhibitor of Cdc25A, induces extracellular signal-regulated kinase 1/2 phosphorylation and inhibits hepatocellular carcinoma growth in vitro and in vivo, *Mol Cancer Ther.* 2005, 4:1378-87, or Brezak *et. al.*, A Novel Synthetic Inhibitor of CDC25 Phosphatases: BN82002, *Cancer Res.* 2004, 64(9):3320-5).

The present invention provides compositions and methods for treating cancer in a patient. This invention is based on the discovery that combinations of CDK inhibitors and CDC25A inhibitors are effective in the treatment of a variety of forms of cancer.

Therapeutic Methods, Combinations and Uses

Combination therapy has become increasingly important for the treatment of cancer patients. The goal of combination therapy is to achieve an additive or synergistic effect between therapeutic agents, thereby facilitating shortened treatment times, decreased toxicity, and/or increased patient survival. Furthermore, treatment of certain types of cancer can be hindered by either pre-existing resistance in the *de novo* cancer cells and/or the development of resistance in the cancer cells to anti-cancer agents used to treat that cancer.

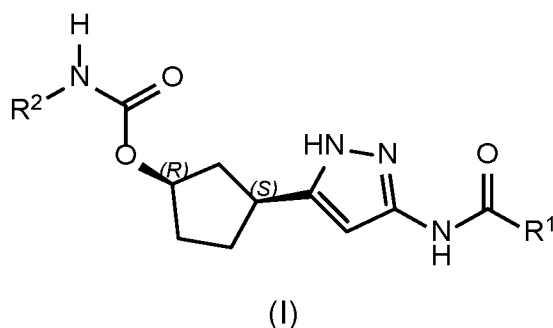
The present invention therefore seeks to provide a new combination of therapeutic agents that is suitable for the treatment of cancer. More specifically, the present invention centers on the surprising and unexpected effects associated with using certain therapeutic agents in combination. In particular, the present invention provides therapeutic methods and uses comprising administering to the subject a therapy that comprises compounds of the present invention alone or in combination with other therapeutic agents.

In some embodiments of each of the methods, combinations and uses described herein, an amount of a first compound or component is combined with an amount of a second compound or component, and the amounts together are effective in the treatment of cancer. The amounts, which together are effective, will relieve to some extent one or more of the symptoms of the disorder being treated. In reference to the treatment of cancer, an effective amount refers to that amount which has the effect of (1) reducing the size of the tumor, (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis emergence, (3) inhibiting to some extent (that is, slowing to some extent, preferably stopping) tumor growth or tumor invasiveness, and/or (4) relieving to some extent (or, preferably, eliminating) one or more signs or symptoms associated with the cancer. Therapeutic or pharmacological effectiveness of the doses and administration regimens may also be characterized as the ability to induce, enhance, maintain or prolong disease control and/or overall survival in patients with these specific tumors, which may be measured as prolongation of the time before disease progression".

In some embodiments of each of the methods, combinations and uses described herein, the present invention comprise a CDK inhibitor. CDKs and related serine/threonine kinases are important cellular enzymes that perform essential functions in regulating cell division and proliferation.

In one aspect, the present invention provides for treating cancer comprising administering to a subject in need thereof an amount of a cyclin dependent kinase (CDK) inhibitor in combination with a CDC25A inhibitor.

In some embodiments of each of the methods, combinations and uses described herein, the CDK inhibitor is an inhibitor of CDK2 (CDK2 inhibitor). In one embodiment, the CDK2 inhibitor is an inhibitor of the CDK2 inhibitor is a compound of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

R¹ is -L-(5-6 membered heteroaryl) or -L-(phenyl), where said 5-6 membered heteroaryl or phenyl is optionally substituted by one to three R³;

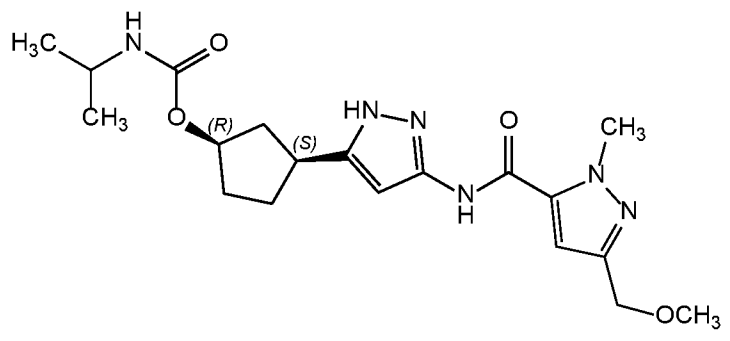
R² is C₁-C₆ alkyl or C₃-C₇ cycloalkyl, where said C₃-C₇ cycloalkyl is optionally substituted by C₁-C₄ alkyl;

L is a bond or a methylene; and

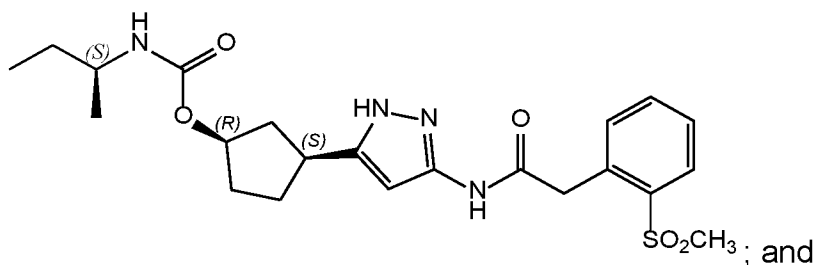
each R³ is independently C₁-C₄ alkyl, C₁-C₄ alkoxy or SO₂-C₁-C₄ alkyl, where each C₁-C₄ alkyl is optionally substituted by F, OH or C₁-C₄ alkoxy.

In some embodiments of each of the methods, combinations and uses described herein, the CDK2 inhibitor is selected from the group consisting of:

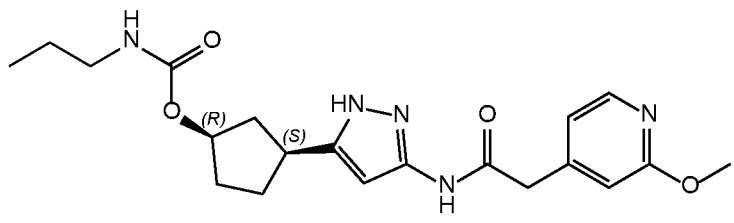
(1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A), having the structure:



(1*R*,3*S*)-3-[3-({[2-(methylsulfonyl)phenyl]acetyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate (COMPOUND B), having the structure:

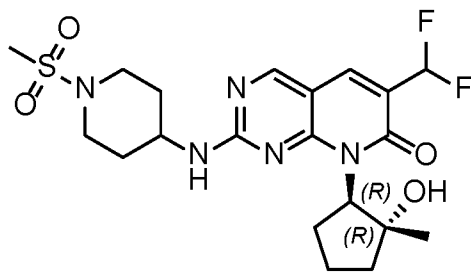


(1*R*,3*S*)-3-(3-[[2-methoxypyridin-4-yl]acetyl]amino)-1*H*-pyrazol-5-yl)cyclopentyl propylcarbamate (COMPOUND C), having the structure:



5 or a pharmaceutically acceptable salt thereof.

In some embodiments of each of the methods, combinations and uses described herein, the CDK2 inhibitor is an inhibitor of CDK2, CDK4 and CDK6 (CDK2/4/6 inhibitor or CDK2/4/6i). In some such embodiments, the CDK2/4/6 inhibitor is 6-(difluoromethyl)-8-((1*R*,2*R*)-2-hydroxy-2-methylcyclopentyl)-2-(1-(methylsulfonyl)piperidin-4-ylamino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF-06873600), having the structure:



or a pharmaceutically acceptable salt thereof.

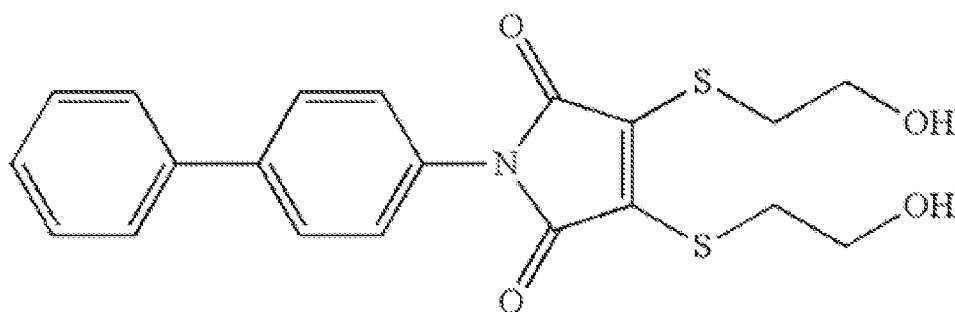
PF-06873600 and pharmaceutically acceptable salts thereof, are disclosed in International Publication No. WO 2018/033815 published February 22, 2018. The contents of that reference are incorporated herein by reference in their entirety.

In some embodiments of each of the methods, combinations and uses described herein, the present invention comprise an inhibitor of cell division cycle 25 A (CDC25A), (CDC25A inhibitor).

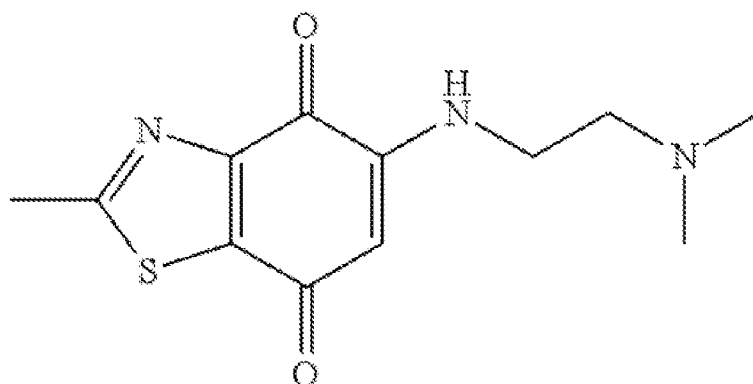
In some embodiments of each of the methods, combinations and uses described herein, the CDC25A phosphatase inhibitor that may be used according to the present invention is PM-20 (i.e., 1-([1,1'-biphenyl]-4-yl)-3,4-bis((2-hydroxyethyl)thio)-1*H*-pyrrole-2,5-dione), a maleimide derivative described in WO 2005/081972, EP1722781, US2008039518 and US7,504,430). PM-20 and its derivatives induce tyrosine

phosphorylation of EGFR and extracellular signal-regulated kinase, which are directly connected to the inhibition of tumor cell growth *in vitro* (Kar S, *et al.*, PM-20, a novel inhibitor of Cdc25A, induces extracellular signal-regulated kinase 1/2 phosphorylation and inhibits hepatocellular carcinoma growth *in vitro* and *in vivo*, *Mol Cancer Ther.* 2006, 5:1511–9). Cell cycle analysis reveals a block, mainly in the G1 phase of the cell cycle, which results in upregulation of CDK1, 2 and 4 tyrosine phosphorylation. When delivered intraperitoneally, PM-20 also inhibits the growth of transplanted rat hepatoma cells (Fisher F344) *in vivo* (Kar S, 2006).

In some embodiments of each of the methods, combinations and uses described herein, the CDC25A inhibitor is PM-20, disclosed in WO 2005/081972:



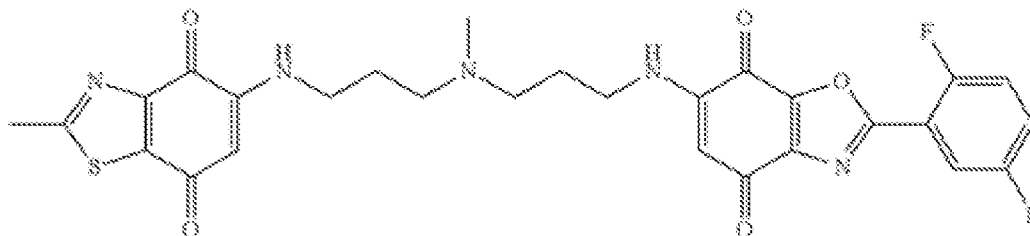
In some embodiments of each of the methods, combinations and uses described herein, the CDC25A phosphatase inhibitor is the quinone derivative BN82685 (i.e., 5-((2-(dimethylamino)ethyl)amino)-2-methylbenzo[d]thiazole-4,7-dione) a quinone-based CDC25 inhibitor described by Brezak, 2005):



The effect of BN82685 on CDC25 phosphatases is shown by an increase in phosphorylation on the tyrosine 15 residue of CDK1. BN82685 irreversibly inhibits the activity of purified recombinant CDC25A, B and C with comparable potency (IC₅₀=0.250, 0.250 and 0.171 μM, respectively).

In some embodiments of each of the methods, combinations and uses described herein, the CDC25A phosphatase inhibitor the quinone derivative IRC 083864, (i.e., 2-(2,5-difluorophenyl)-6-((3-(methyl(3-((2-methyl-4,7-dioxo-4,7-dihydrobenzo[d]thiazol-5-

yl)amino)propyl)amino)propyl)amino)benzo[d]oxazole-4,7-dione; WO2006/067311; EP1831209; JP2008524175):



In some embodiments of each of the methods, combinations and uses described herein, the CDC25A phosphatase inhibitor include any of the CDC25A phosphatase inhibitor described in Lavecchia *et al.*, Inhibitors of Cdc25 Phosphatases as Anticancer Agents: A Patent Review, *Exp. Opin. Ther. Pat.* 2010, 20(3):405-425), all of which are herein incorporated by reference.

In some embodiments of each of the methods, combinations and uses described herein, IRC 083864 is a CDC25A phosphatase inhibitor. The dose used for IRC 083864 is from 1 to 10 μM , preferably from 2.5 μM to 5 μM .

In some embodiments of each of the methods, combinations and uses described herein, the inhibition of the phosphorylation of the CDC25A substrate in the presence of the inhibitor may be observed in a dose-dependent manner and the measured signal is at least 10 percent lower, preferably at least 50 percent lower than the signal measured with a negative control under comparable conditions. The CDC25A inhibitor of the present the present invention exhibits an IC_{50} of at least 1 μM , preferably 100 nM as measured in at least one of the assays described above.

According to the present invention, the CDC25A inhibitor provides the advantage of inhibiting proliferation and re-inducing differentiation of tumor cells which are valuable for the treatment of drug resistant cancer and/or for use in the prevention of a tumor relapse in a patient suffering or having suffered from cancer.

In some embodiments of each of the methods, combinations and uses described herein, the CDC25A inhibitor is the inhibitor of CDC25A phosphatase expression for use in the combination therapy as described herein. For example, the CDC25A inhibitor is administered in combination with CDK2 inhibitor for the treatment of drug resistant cancer in a patient suffering from cancer and/or for use in the prevention of tumor relapse in a patient who suffers or has suffered from cancer.

In some embodiments of each of the methods, combinations and uses described herein, the inhibitors of CDC25A phosphatase expression are based on anti-sense

oligonucleotide constructs, as described herein. Anti-sense oligonucleotides, including anti-sense RNA molecules and anti-sense DNA molecules, act to directly block the translation of CDC25A phosphatase mRNA by binding thereto and thus preventing protein translation or increasing mRNA degradation, thus decreasing the level of CDC25A phosphatase proteins, and CDC25A activity, in a cell. For example, anti-sense oligonucleotides of at least about 15 bases and complementary to unique regions of the mRNA transcript sequence encoding CDC25A phosphatase may be synthesized, e.g., by conventional phosphodiester techniques and administered by e.g., intravenous injection or infusion. Methods for using antisense techniques for specifically inhibiting gene expression of genes whose sequence is known are well known in the art (e.g., U.S. Pat. Nos. 6,566,135; 6,566,131; 6,365,354; 6,410,323; 6,107,091; 6,046,321; and 5,981,732).

In some embodiments of each of the methods, combinations and uses described herein, inhibitors of CDC25A phosphatase expression are small inhibitory RNAs (siRNAs), as described herein. CDC25A phosphatase gene expression may be reduced by contacting the tumor, subject or cell with a small double stranded RNA (dsRNA), or a vector or construct causing the production of a small double stranded RNA, such that CDC25A phosphatase expression is specifically inhibited (i.e., RNA interference or RNAi). Methods for selecting an appropriate dsRNA or dsRNA-encoding vector are well known in the art for genes whose sequence is known (e.g., Tuschl, T. *et al.*, Targeted mRNA Degradation by Double-Stranded RNA in Vitro, *Genes Dev.* 1999, 13(24):3191-7; Elbashir, S.M. *et al.* Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 411, 2001, 494-498; Hannon, Gregory J., RNA Interference, *Nature*, 2002, vol. 418: 244-251; McManus M T and Sharp P A, Gene silencing in mammals by small interfering RNAs, *Nature Rev Genet* 2002, 3: 737-747; Brummelkamp TR, *et al.*, A system for stable expression of short interfering RNAs in mammalian cells, *Science* 2002, 296(5567): 550-553; U.S. Pat. Nos. 6,573,099 and 6,506,559; and International Patent Publication Nos. WO 2001/036646, WO 1999/032619, and WO 2001/068836).

In some embodiments of each of the methods, combinations and uses described herein, inhibitors of CDC25A phosphatase expression are ribozymes, as described herein. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Engineered hairpin or hammerhead motif ribozyme molecules

that specifically and efficiently catalyze endonucleolytic cleavage of CDC25A phosphatase mRNA sequences are thereby useful within the scope of the present invention. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which typically include the following sequences, GUA, GUU, and GUC. Once identified, short RNA sequences of between about 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features, such as secondary structure, that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using, e.g., ribonuclease protection assays.

Both antisense oligonucleotides and ribozymes useful as inhibitors of CDC25A phosphatase expression may be prepared by known methods. These include techniques for chemical synthesis such as, e.g., by solid phase phosphoramidite chemical synthesis. Alternatively, anti-sense RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences encoding the RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Various modifications to the oligonucleotides of the present invention may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the oligonucleotide backbone.

In some embodiments of each of the methods, combinations and uses described herein, the compounds and methods described herein can be used to treat a subject suffering from cancer wherein the cancer is characterized by loss of RB. In some such embodiments, the cancer is breast cancer, small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNEC), or neuroendocrine prostate cancer (NEPC).

Pharmaceutical compositions suitable for the delivery of compounds of the present invention, meaning CDK inhibitors and CDC25A inhibitors as described herein, and methods for their preparation will be readily apparent to those skilled in the art. Such compositions and methods for their preparation can be found, for example, in Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company (1995), Cover and pp. 1660, 1662, 1664 and 1665 of Chapter 94), the disclosure of which is incorporated herein by reference in its entirety.

The CDK2 and CDC25A inhibitors may be administered orally. Oral administration may involve swallowing, so that the compound enters the gastrointestinal tract, or buccal or sublingual administration may be employed by which the compound enters the blood stream directly from the mouth.

5 Formulations suitable for oral administration include solid formulations such as tablets, capsules containing particulates, liquids, or powders, lozenges (including liquid-filled), chews, multi- and nano-particulates, gels, solid solution, liposome, films (including muco-adhesive), ovules, sprays and liquid formulations.

10 Liquid formulations include suspensions, solutions, syrups and elixirs. Such formulations may be used as fillers in soft or hard capsules and typically include a carrier, for example, water, ethanol, polyethylene glycol, propylene glycol, methylcellulose, or a suitable oil, and one or more emulsifying agents and/or suspending agents. Liquid formulations may also be prepared by the reconstitution of a solid, for example, from a sachet.

15 The CDK2 and CDC25A inhibitors may also be used in fast-dissolving, fast-disintegrating dosage forms such as those described in Liang et al., Fast-dissolving intraoral drug delivery systems, Expert Opinion in Therapeutic Patents 2001, 11(6), 981-986, the disclosure of which is incorporated herein by reference in its entirety.

20 For tablet dosage forms, depending on dose, the drug may make up from 1 wt% to 80 wt% of the dosage form, more typically from 5 wt% to 60 wt% of the dosage form. In addition to the drug, tablets generally contain a disintegrant. Examples of disintegrants include sodium starch glycolate, sodium carboxymethyl cellulose, calcium carboxymethyl cellulose, croscarmellose sodium, crospovidone, polyvinylpyrrolidone, methyl cellulose, microcrystalline cellulose, lower alkyl-substituted hydroxypropyl cellulose, starch, 25 pregelatinized starch and sodium alginate. Generally, the disintegrants will comprise from 1 wt% to 25 wt%, preferably from 5 wt% to 20 wt% of the dosage form.

30 Binders are generally used to impart cohesive qualities to a tablet formulation. Suitable binders include microcrystalline cellulose, gelatin, sugars, polyethylene glycol, natural and synthetic gums, polyvinylpyrrolidone, pregelatinized starch, hydroxypropyl cellulose and hydroxypropyl methylcellulose. Tablets may also contain diluents, such as lactose (monohydrate, spray-dried monohydrate, anhydrous and the like), mannitol, xylitol, dextrose, sucrose, sorbitol, microcrystalline cellulose, starch and dibasic calcium phosphate dihydrate.

35 Tablets may also optionally include surface active agents, such as sodium lauryl sulfate and polysorbate 80, and glidants such as silicon dioxide and talc. When present,

surface active agents are typically in amounts of from 0.2 wt% to 5 wt% of the tablet, and glidants typically from 0.2 wt% to 1 wt% of the tablet. Tablets also generally contain lubricants such as magnesium stearate, calcium stearate, zinc stearate, sodium stearyl fumarate, and mixtures of magnesium stearate with sodium lauryl sulphate. Lubricants
5 generally are present in amounts from 0.25 wt% to 10 wt%, preferably from 0.5 wt% to 3 wt% of the tablet.

Other conventional ingredients include antioxidants, colorants, flavoring agents, preservatives and taste-masking agents.

Exemplary tablets contain up to about 80 wt% drug, from about 10 wt% to about
10 90 wt% binder, from about 0 wt% to about 85 wt% diluent, from about 2 wt% to about 10 wt% disintegrant, and from about 0.25 wt% to about 10 wt% lubricant.

Tablet blends may be compressed directly or by roller to form tablets. Tablet blends or portions of blends may alternatively be wet-, dry-, or melt-granulated, melt congealed, or extruded before tableting. The final formulation may include one or more
15 layers and may be coated or uncoated; or encapsulated.

The formulation of tablets is discussed in detail in Doelker, *et al.*, Pharmaceutical dosage forms: Tablets, *Journal of Controlled Release* 1991, Vol 15:2 185 the disclosure of which is incorporated herein by reference in its entirety. Solid formulations for oral administration may be formulated to be immediate and/or modified release. Modified
20 release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release. Suitable modified release formulations are described in U.S. Patent No. 6,106,864. Details of other suitable release technologies such as high energy dispersions and osmotic and coated particles can be found in Verma RK, Garg S. Current status of drug delivery technologies and future directions. *Pharmaceutical Technology*.
25 2001;25(2):1–14. The use of chewing gum to achieve controlled release is described in WO 2000/035298. The disclosures of these references are incorporated herein by reference in their entireties.

In some embodiments of each of the methods, combinations and uses described herein, the CDK2 and CDC25A inhibitors of the present invention may be administered
30 directly into the blood stream, into muscle, or into an internal organ. Suitable means for parenteral administration include intravenous, intraarterial, intraperitoneal, intrathecal, intraventricular, intraurethral, intrasternal, intracranial, intramuscular and subcutaneous. Suitable devices for parenteral administration include needle (including micro needle) injectors, needle-free injectors and infusion techniques.

Parenteral formulations are typically aqueous solutions which may contain excipients such as salts, carbohydrates and buffering agents (preferably to a pH of from 3 to 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water.

The preparation of parenteral formulations under sterile conditions, for example, by lyophilization, may readily be accomplished using standard pharmaceutical techniques well known to those skilled in the art. The solubility of compounds of the present invention used in the preparation of parenteral solutions may be increased using appropriate formulation techniques, such as the incorporation of solubility-enhancing agents. Formulations for parenteral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release. Thus, compounds of the present invention may be formulated as a solid, semi-solid, or thixotropic liquid for administration as an implanted depot providing modified release of the active compound. Examples of such formulations include drug-coated stents and PGLA microspheres.

In some embodiments of each of the methods, combinations and uses described herein, the CDK2 and CDC25A inhibitors of the present invention may be administered topically to the skin or mucosa, that is, dermally or transdermally. Typical formulations for this purpose include gels, hydrogels, lotions, solutions, creams, ointments, dusting powders, dressings, foams, films, skin patches, wafers, implants, sponges, fibers, bandages and microemulsions. Liposomes may also be used. Typical carriers include alcohol, water, mineral oil, liquid petrolatum, white petrolatum, glycerin, polyethylene glycol and propylene glycol. Penetration enhancers may be incorporated; see, for example, Finnin, *et al.*, Transdermal penetration enhancers: Applications, limitations, and potential, *Journal of Pharm. Sci.* 1999, Vol 88:10 PP 955-958. Other means of topical administration include delivery by electroporation, iontophoresis, phonophoresis, sonophoresis and micro needle or needle-free (e.g. Powderject™, Bioject™, etc.) injection. The disclosures of these references are incorporated herein by reference in their entireties. Formulations for topical administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

In some embodiments of each of the methods, combinations and uses described herein, the CDK2 and CDC25A inhibitors of the present invention may be administered intranasally or by inhalation, typically in the form of a dry powder (either alone, as a

mixture, for example, in a dry blend with lactose, or as a mixed component particle, for example, mixed with phospholipids, such as phosphatidylcholine) from a dry powder inhaler or as an aerosol spray from a pressurized container, pump, spray, atomizer (preferably an atomizer using electrohydrodynamics to produce a fine mist), or nebulizer, with or without the use of a suitable propellant known within the art. For intranasal use, the powder may include a bioadhesive agent, for example, chitosan or cyclodextrin.

The pressurized container, pump, spray, atomizer, or nebulizer contains a solution or suspension of the compound(s) of the present invention comprising, for example, ethanol, aqueous ethanol, or a suitable alternative agent for dispersing, solubilizing, or extending release of the active, a propellant(s) as solvent and an optional surfactant, such as sorbitan trioleate, oleic acid, or an oligolactic acid. Prior to use in a dry powder or suspension formulation, the drug product is micronized to a size suitable for delivery by inhalation (typically less than 5 microns). This may be achieved by any appropriate comminuting method, such as spiral jet milling, fluid bed jet milling, supercritical fluid processing to form nanoparticles, high pressure homogenization, or spray drying.

Capsules (made, for example, from gelatin or HPMC), blisters and cartridges for use in an inhaler or insufflator may be formulated to contain a powder mix of the CDK2 inhibitors, CDC25A inhibitors, a suitable powder base such as lactose or starch and a performance modifier such as L-leucine, mannitol, or magnesium stearate. The lactose may be anhydrous or in the form of the monohydrate, preferably the latter. Other suitable excipients include dextran, glucose, maltose, sorbitol, xylitol, fructose, sucrose and trehalose.

A suitable solution formulation for use in an atomizer using electrohydrodynamics to produce a fine mist may contain from 1 µg to 20mg of the CDK2 and CDC25A inhibitors of the present invention per actuation and the actuation volume may vary from 1 µL to 100µL. A typical formulation includes one or more CDK2 inhibitors of the present invention, propylene glycol, sterile water, ethanol and sodium chloride. A formulation of the present invention may include one or more CDC25A inhibitors, propylene glycol, sterile water, ethanol and sodium chloride. Alternative solvents which may be used instead of propylene glycol include glycerol and polyethylene glycol. Suitable flavors, such as menthol and levomenthol, or sweeteners, such as saccharin or saccharin sodium, may be added to those formulations of the present invention intended for inhaled/intranasal administration.

Formulations for inhaled/intranasal administration may be formulated to be immediate and/or modified release using, for example, poly(DL-lactic-coglycolic acid

(PGLA). Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

In the case of dry powder inhalers and aerosols, the dosage unit is determined by means of a valve which delivers a metered amount. Units in accordance with the present invention are typically arranged to administer a metered dose or "puff" containing, preferably, a desired amount of CDK2 and CDC25A inhibitors of the present invention. The overall daily dose may be administered in a single dose or, more usually, as divided doses throughout the day.

In some embodiments of each of the methods, combinations and uses described herein, the CDK2 and CDC25A inhibitors of the present invention may be administered rectally or vaginally, for example, in the form of a suppository, pessary, or enema. Cocoa butter is a traditional suppository base, but various alternatives may be used as appropriate. Formulations for rectal/vaginal administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

In some embodiments of each of the methods, combinations and uses described herein, the CDK2 and CDC25A inhibitors of the present invention may be administered directly to the eye or ear, typically in the form of drops of a micronized suspension or solution in isotonic, pH adjusted, sterile saline. Other formulations suitable for ocular and aural administration include ointments, biodegradable (e.g. absorbable gel sponges, collagen) and non-biodegradable (e.g. silicone) implants, wafers, lenses and particulate or vesicular systems, such as niosomes or liposomes. A polymer such as cross-linked polyacrylic acid, polyvinylalcohol, hyaluronic acid, a cellulosic polymer, for example, hydroxypropylmethylcellulose, hydroxyethylcellulose, or methyl cellulose, or a heteropolysaccharide polymer, for example, gelatin, may be incorporated together with a preservative, such as benzalkonium chloride. Such formulations may also be delivered by iontophoresis. Formulations for ocular/aural administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted, or programmed release.

In some embodiments of each of the methods, combinations and uses described herein, the CDK2 and CDC25A inhibitors of the present invention may be combined with soluble macromolecular entities, such as cyclodextrin and suitable derivatives thereof, or polyethylene glycol-containing polymers, in order to improve their solubility, dissolution rate, taste-masking, bioavailability and/or stability for use in any of the modes of administration.

Drug-cyclodextrin complexes, for example, are found to be generally useful for most dosage forms and administration routes. Both inclusion and non-inclusion complexes may be used. As an alternative to direct complexation with the drug, the cyclodextrin may be used as an auxiliary additive, *i.e.*, as a carrier, diluent, or solubilizer. Most commonly used for these purposes are alpha-, beta- and gamma-cyclodextrins, examples of which may be found in PCT Publication Nos. WO 1991/011172, WO 1994/002518 and WO 1998/055148, the disclosures of which are incorporated herein by reference in their entireties.

Inasmuch as it may be desirable to administer a combination of CDK2 and/or CDC25A inhibitors individually or collectively, for example, for the purpose of treating a particular disease or condition, it is within the scope of the present invention that pharmaceutical compositions, at least one of which contains at least one CDK2 and/or CDC25A inhibitor, or a combination of CDK2 and/or CDC25A inhibitors in accordance with the present invention, may conveniently be combined in the form of a kit suitable for co-administration of the compositions. Thus, the kit of the present invention includes two or more separate pharmaceutical compositions, at least one of which contains a CDK2 and/or CDC25A inhibitor of the present invention, and means for separately retaining said compositions, such as a container, divided bottle, or divided foil packet. An example of such a kit is the familiar blister pack used for the packaging of tablets, capsules and the like. The kit of the present invention is particularly suitable for administering different dosage forms, for example, oral and parenteral, for administering the separate compositions at different dosage intervals, or for titrating the separate compositions against one another. To assist compliance, the kit typically includes directions for administration and may be provided with a memory aid.

In some embodiments of each of the methods, combinations and uses herein, the CDK2 inhibitor and/or CDC25A inhibitor of the present invention may be part of a combination therapy. As used herein, the term "combination therapy" refers to the administration of a CDK2 inhibitor and a CDC25A inhibitor of the present invention together with at least one additional pharmaceutical or medicinal agent (*e.g.*, an anti-cancer agent), either simultaneously, separately or sequentially, as mixed or individual dosages. As noted above, the CDK2 and CDC25A inhibitors of the present invention may be used in combination with each other, and in some embodiment one or more additional anti-cancer agents. The efficacy of the compounds of the present invention in certain tumors may be enhanced by combination with other approved or experimental cancer therapies, *e.g.*, radiation, surgery, chemotherapeutic agents, targeted therapies,

agents that inhibit other signaling pathways that are dysregulated in tumors, and other immune enhancing agents, such as PD-1 antagonists and the like.

In some embodiments of each of the methods, combinations and uses herein, the breast cancer is optionally node negative or node positive, estrogen receptor (ER) positive (ER+) or estrogen receptor (ER) negative (ER-), HER2 positive or HER2 negative, PR positive or PR negative, high grade or low grade, basal-like or luminal-like, or a combination of these factors.

Breast cancers that have estrogen receptors are called ER-positive (or ER+) cancers. Breast cancers with progesterone receptors are called PR-positive (or PR+) cancers.

Triple-negative breast cancer cells don't have estrogen or progesterone receptors and also don't make too much of the protein called HER2 (ER/PR/HER2 negative).

In some embodiments of each of the methods, combinations and uses herein, the combination therapy can be used to treat a subject suffering from an RB positive (RB+) cancer or other RB positive abnormal cellular proliferative disorders. Cancers and disorders of such type can be characterized by (e.g., that has cells that exhibit) the presence of a functional retinoblastoma protein. Such cancers and disorders are classified as being RB positive. RB positive abnormal cellular proliferation disorders, and variations of this term as used herein, refer to disorders or diseases caused by uncontrolled or abnormal cellular division which are characterized by the presence of a functional retinoblastoma protein, which can include cancers. In one embodiment of the present invention, the compounds and methods described herein can be used to treat a non-cancerous RB positive abnormal cellular proliferation disorder. Examples of such disorders may include non-malignant lymphoproliferation, non-malignant breast neoplasms, psoriasis, arthritis, dermatitis, pre-cancerous colon lesions or pulps, angiogenesis disorders, immune mediated and non-immune mediated inflammatory diseases, arthritis, age-related macular degeneration, diabetes, and other non-cancerous or benign cellular proliferation disorders.

In some embodiments of each of the methods, combinations and uses herein, the RB positive cancer can be RB positive adenocarcinoma. The RB positive cancer can be RB positive adenocarcinoma of the colon. The RB positive cancer can also be RB positive adenocarcinoma of the rectum.

Alternatively, the RB positive cancer can be an RB positive anaplastic astrocytoma. The RB positive cancer can be RB positive breast cancer. In one embodiment, the RB positive cancer is RB positive estrogen-receptor positive, HER2-

negative advanced breast cancer. Alternatively, the RB positive cancer can be RB positive estrogen receptor-negative breast cancer. The RB positive cancer can be RB positive estrogen receptor positive breast cancer. The RB positive cancer can be RB positive late-line metastatic breast cancer. The RB positive cancer can be RB positive luminal A breast cancer. The RB positive cancer can be RB positive luminal B breast cancer. The RB positive cancer can be RB positive Her2 -negative breast cancer or RB positive HER2 -positive breast cancer. The RB positive cancer is RB positive male breast cancer. In one embodiment, the RB positive cancer is RB positive progesterone receptor-negative breast cancer. The RB positive cancer can be RB positive progesterone receptor-positive breast cancer. The RB positive cancer can be RB positive recurrent breast cancer. In one embodiment, the RB positive cancer is RB positive stage IV breast cancers. In one embodiment, the RB positive cancer is RB positive advanced HER2 -positive breast cancer.

The RB positive cancer can be RB positive bronchial cancer. The RB positive cancer can be RB positive colorectal cancer. The RB positive cancer can be RB positive recurrent colorectal cancer. The RB positive cancer can be RB positive stage IV colorectal cancers. In one embodiment, the RB positive cancer is RB positive colorectal cancer.

In one embodiment, the RB positive cancer is RB positive endometrial cancer.

The RB positive cancer can be RB positive extragonadal seminoma. The RB positive cancer can be RB positive stage III extragonadal seminoma. The RB positive cancer can be RB positive stage IV extragonadal seminoma. The RB positive cancer can be RB positive germ cell cancer. The RB positive cancer can be RB positive central nervous system germ cell tumor. The RB positive cancer can be RB positive familial testicular germ cell tumor. The RB positive cancer can be RB positive recurrent gonadal germ cell tumor. The RB positive cancer can be RB positive recurrent extragonadal non-seminomatous germ cell tumor. The RB positive cancer can be RB positive extragonadal seminomatous germ cell tumor. The RB positive cancer can be RB positive recurrent malignant testicular germ cell tumors. The RB positive cancer can be RB positive recurrent ovarian germ cell tumors. The RB positive cancer can be RB positive stage III malignant testicular germ cell tumors. The RB positive cancer can be RB positive stage III ovarian germ cell tumors. The RB positive cancer can be RB positive stage IV ovarian germ cell tumors. The RB positive cancer can be RB positive stage III extragonadal non-seminomatous germ cell tumors. The RB positive cancer can be RB positive stage IV extragonadal non-seminomatous germ cell tumors. In one embodiment, the RB positive

cancer is RB positive germ cell cancer. In one embodiment, the RB positive cancer is RB positive cisplatin-refractory, unresectable germ cell cancer.

In one embodiment, the RB positive cancer is RB positive glioblastoma.

In one embodiment, the RB positive cancer is RB positive liver cancer. The RB
5 positive cancer can be RB positive hepatocellular cancer.

The RB positive cancer can be RB positive lung cancer. In one embodiment, the RB positive cancer is RB positive non-small cell lung cancer. In one embodiment, the RB positive cancer is RB positive KRAS mutant non-small cell lung cancer.

The RB positive cancer can be RB positive melanoma. In one embodiment, the
10 RB positive cancer is RB positive recurrent melanomas. In one embodiment, the RB positive cancer is RB positive stage IV melanomas.

The RB positive cancer can be RB positive ovarian cancer. In one embodiment, the RB positive cancer is RB positive ovarian epithelial carcinoma.

The RB positive cancer can be RB positive pancreatic cancer.

15 In one embodiment, the RB positive cancer is RB positive rectal cancer. The RB positive cancer can be RB positive recurrent rectal cancer. The RB positive cancer can be RB positive stage IV rectal cancers. The RB positive cancer can be RB positive sarcoma. The RB positive cancer can be RB positive gliosarcoma. The RB positive cancer can be RB positive liposarcoma. The RB positive cancer can be RB positive
20 fibrosarcoma. The RB positive cancer can be RB positive myxosarcoma. In one embodiment, the RB positive cancer can be RB positive chondrosarcoma. The RB positive cancer can be RB positive osteosarcoma.

The RB positive cancer can be RB positive malignant fibrous histiocyoma. The RB positive cancer can be RB positive hemangiosarcoma. The RB positive cancer can
25 be RB positive angiosarcoma. The RB positive cancer can be RB positive lymphangiosarcoma. The RB positive cancer can be RB positive mesothelioma. The RB positive cancer can be RB positive leiomyosarcoma. The RB positive cancer can be RB positive rhabdomyosarcoma. The RB positive cancer can be an RB positive meningioma. The RB positive cancer can be an RB positive schwannoma.

30 In one embodiment, the RB positive cancer is an RB positive pheochromocytoma. The RB positive cancer can be an RB positive Islet cell carcinoma. The RB positive cancer can be RB positive carcinoid. The RB positive cancer can be an RB positive paraganglioma.

In one embodiment, the RB positive cancer is RB positive squamous cell
35 carcinoma. The RB positive cancer can be RB positive adenocarcinoma. The RB

positive cancer can be RB positive hepatocellular carcinoma. The RB positive cancer can be RB positive renal cell carcinoma. The RB positive cancer can be RB positive cholangiocarcinoma.

The RB positive cancer can be RB positive refractory solid tumors.

5 The RB positive cancer can be RB positive neuroblastoma.

The RB positive cancer can be RB positive medulloblastoma.

In one embodiment, the RB positive cancer is a Teratoma. The RB positive cancer can be RB positive ovarian immature teratoma. The RB positive cancer can be an RB positive ovarian mature teratoma. The RB positive cancer can be an RB positive ovarian
10 specialized teratoma. The RB positive cancer can be RB positive testicular immature teratoma. The RB positive cancer can be RB positive testicular mature teratoma. The RB positive cancer can be RB positive teratoma. The RB positive cancer can be RB positive ovarian monodermal teratoma.

The RB positive cancer can be RB positive testicular cancer.

15 In one embodiment, the RB positive cancer is RB positive vaginal cancer. In one embodiment, the RB positive cancer is selected from an RB positive carcinoma, sarcoma, including, but not limited to, lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colorectal cancer,
20 breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer
25 of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain stem glioma, pituitary adenoma, or a combination of one or more of the foregoing cancers.

In a specific embodiment, the RB positive cancer is an RB positive prostate cancer.

30 In another specific embodiment, the RB positive cancer is an RB positive SCLC cancer.

In one embodiment, the subject is suffering from an RB positive abnormal cellular proliferation disorder. In one embodiment, the RB positive abnormal cellular proliferation disorder is non-cancerous. In certain embodiments, a compound described herein, when used to treat a select RB positive cellular proliferation disorder, such as a cancer, allows
35 for a rapid reentry of healthy cells into the normal cell-cycle and a fast reconstitution of

damaged tissue and progeny cells such as hematological cells. In this aspect, the compounds described herein when used to treat RB positive cancers eliminate, reduce, and/or minimize the drug holidays and dose delays associated with the current anti-neoplastic use of CDK2 inhibitors, allowing for the quick recovery of damaged blood cells through the replication and differentiation of progenitor and parent cells. Specifically, the present invention includes administering to a patient having a cancer such as an RB positive cancer an effective amount of a compound described herein, wherein the compound has a pharmacokinetic and enzymatic half-life that provides for a transient, reversible G1 arrest of CDK2-replication dependent cells. The compound can be any of those described in this application. Non-limiting examples of active compounds are described herein, or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof as provided below.

The presence or normal functioning of the retinoblastoma (RB) tumor suppressor protein (RB positive or RB+) can be determined through any of the standard assays known to one of ordinary skill in the art, including but not limited to Western Blot, ELISA (enzyme linked immunoadsorbent assay), IHC (immunohistochemistry), and FACS (fluorescent activated cell sorting). The selection of the assay will depend upon the tissue, cell line or surrogate tissue sample that is utilized e.g., for example Western Blot and ELISA may be used with any or all types of tissues, cell lines or surrogate tissues, whereas the IHC method would be more appropriate wherein the tissue utilized in the methods of the present invention was a tumor biopsy. FACS analysis would be most applicable to samples that were single cell suspensions such as cell lines and isolated peripheral blood mononuclear cells. See for example, US 2007/212736 "Functional Immunohistochemical Cell Cycle Analysis as a Prognostic Indicator for Cancer". Alternatively, molecular genetic testing may be used for determination of retinoblastoma gene status. Lohmann and Gallie, Molecular genetic testing for retinoblastoma includes the following as described, *Retinoblastoma. Gene Reviews* 2010, <http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=retinoblastoma> or Parsam *et al.*, A comprehensive, sensitive and economical approach for the detection of mutations in the RB 1 gene in retinoblastoma, *Journal of Genetics* 2009, 88(4), 517-527.

In some embodiments of each of the methods, combinations and uses herein, the present invention relates to a combination therapy useful for the treatment of a subject, preferably a human, that has a cancer associated with cells that are RB deficient or RB negative. In another embodiment, the present invention relates to methods useful for the treatment of a subject, preferable a human, that has a cancer associated with cells that

are RB positive. In some such embodiments, the cancer is SCLC. In a particular embodiment of each of the foregoing, the method comprises, administering a combination of CDK2 inhibitor and a CDC25A inhibitor as described herein.

The methods, combinations and uses of the present invention may additionally
5 comprise one or more additional anti-cancer agents, such as the anti-angiogenesis agents, signal transduction inhibitors or antineoplastic agents described below, wherein the amounts are together effective in treating cancer. In some embodiments, the methods, combinations and uses of the present invention the additional anti-cancer agents may comprise a palliative care agent. Additional anti-cancer agents may include
10 small molecules therapeutics and pharmaceutically acceptable salts or solvates thereof, therapeutic antibodies, antibody-drug conjugates (ADCs), proteolysis targeting chimeras (PROTACs), or antisense molecules.

In some embodiments, the methods, combinations and uses of the present invention further comprise one or more additional anti-cancer agents selected from the
15 following:

Anti-angiogenesis agents include, for example, VEGF inhibitors, VEGFR inhibitors, TIE-2 inhibitors, PDGFR inhibitors, angiopoietin inhibitors, PKC β inhibitors, COX-2 (cyclooxygenase II) inhibitors, integrins (α -v/ β -3), MMP-2 (matrix-metalloproteinase 2) inhibitors, and MMP-9 (matrix-metalloproteinase 9) inhibitors.

Signal transduction inhibitors include, for example, kinase inhibitors (e.g., inhibitors
20 of tyrosine kinases, serine/threonine kinases or cyclin dependent kinases), proteasome inhibitors, PI3K/AKT/mTOR pathway inhibitors, Phosphoinositide 3-kinase (PI3K) inhibitors, isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) inhibitors, B-cell lymphoma 2 (BCL2) inhibitors, neurotrophin receptor kinase (NTRK) inhibitors, Rearranged during
25 Transfection (RET) inhibitors, Notch inhibitors, PARP inhibitors, Hedgehog pathway inhibitors, and selective inhibitors of nuclear export (SINE).

Examples of signal transduction inhibitors include, but are not limited to:
acalabrutinib, afatinib, alectinib, alpelisib, axitinib, binimetinib, bortezomib, bosutinib,
brigatinib, cabozantinib, carfilzomib, ceritinib, cobimetinib, copanlisib, crizotinib,
30 dabrafenib, dacomitinib, dasatinib, duvelisib, enasidenib, encorafenib, entrectinib, erlotinib, gefitinib, gilteritinib, glasdegib, ibrutinib, idelalisib, imatinib, ipatasertib, ivosidenib, ixazomib, lapatinib, larotrectinib, lenvatinib, lorlatinib, midostaurin, neratinib, nilotinib, niraparib, olaparib, osimertinib, pazopanib, ponatinib, regorafenib, rucaparib, ruxolitinib, sonidegib, sorafenib, sunitinib, talazoparib, trametinib, vandetanib,

vemurafenib, venetoclax, and vismodegib, or pharmaceutically acceptable salts and solvates thereof.

Antineoplastic agents include, for example, alkylating agents, platinum coordination complexes, cytotoxic antibiotics, antimetabolites, biologic response
5 modifiers, histone deacetylase (HDAC) inhibitors, hormonal agents, monoclonal antibodies, growth factor inhibitors, taxanes, topoisomerase inhibitors, *Vinca* alkaloids and miscellaneous agents.

Alkylating agents include: altretamine, bendamustine, busulfan, carmustine, chlorambucil, cyclophosphamide, dacarbazine, ifosfamide, lomustine, mechlorethamine,
10 melphalan, procarbazine, streptozocin, temozolomide, thiotepa, and trabectedin.

Platinum coordination complexes include: carboplatin, cisplatin, and oxaliplatin.

Cytotoxic antibiotics include: bleomycin, dactinomycin, daunorubicin, doxorubicin, epirubicin, idarubicin, mitomycin, mitoxantrone, plicamycin, and valrubicin.

Antimetabolites include: antifolates, such as methotrexate, pemetrexed,
15 pralatrexate, and trimetrexate; purine analogues, such as azathioprine, cladribine, fludarabine, mercaptopurine, and thioguanine; and pyrimidine analogues such as azacitidine, capecitabine, cytarabine, decitabine, floxuridine, fluorouracil, gemcitabine, and trifluridine/tipiracil.

Biologic response modifiers include: aldesleukin (IL-2), denileukin diftitox, and
20 interferon gamma.

Histone deacetylase inhibitors include belinostat, panobinostat, romidepsin, and vorinostat.

Hormonal agents include antiandrogens, antiestrogens, gonadotropin releasing hormone (GnRH) analogues and peptide hormones. Examples of antiestrogens include:
25 aromatase inhibitors, such as letrozole, anastrozole, and exemestane; SERDs, such as fulvestrant, elacestrant (RAD-1901, Radius Health), SAR439859 (Sanofi), RG6171 (Roche), AZD9833 (AstraZeneca), AZD9496 (AstraZeneca), rintodestrant (G1 Therapeutics), ZN-c5 (Zentalis), LSZ102 (Novartis), D-0502 (Inventisbio), LY3484356 (Lilly), SHR9549 (Jiansu Hengrui Medicine); and SERMs, such as tamoxifen, raloxifene,
30 toremifene, lasofoxifene, bazedoxifene, afimoxifene. Examples of GnRH analogues include: degarelix, goserelin, histrelin, leuprolide, and triptorelin. Examples of peptide hormones include: lanreotide, octreotide, and pasireotide. Examples of antiandrogens include: abiraterone, apalutamide, bicalutamide, cyproterone, enzalutamide, flutamide, and nilutamide, and pharmaceutically acceptable salts and solvates thereof.

Monoclonal antibodies include: alemtuzumab, atezolizumab, avelumab, bevacizumab, blinatumomab, brentuximab, cemiplimab, cetuximab, daratumumab, dinutuximab, durvalumab, elotuzumab, gemtuzumab, inotuzumab, ipilimumab, mogamulizumab, moxetumomab pasudotox, necitumumab, nivolumab, ofatumumab, olaratumab, panitumumab, pembrolizumab, pertuzumab, ramucirumab, rituximab, tositumomab, and trastuzumab.

Taxanes include: cabazitaxel, docetaxel, paclitaxel and paclitaxel albumin-stabilized nanoparticle formulation.

Topoisomerase inhibitors include: etoposide, irinotecan, teniposide, and topotecan.

Vinca alkaloids include: vinblastine, vincristine, and vinorelbine, and pharmaceutically acceptable salts thereof.

Miscellaneous antineoplastic agents include: asparaginase (pegaspargase), bexarotene, eribulin, everolimus, hydroxyurea, ixabepilone, lenalidomide, mitotane, omacetaxine, pomalidomide, tagraxofusp, telotristat, temsirolimus, thalidomide, and venetoclax.

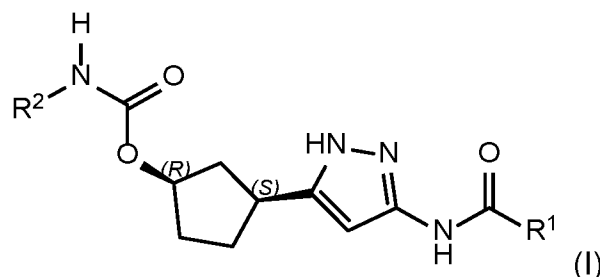
In some embodiments, the additional anti-cancer agent is selected from the group consisting of: abiraterone acetate; acalabrutinib; ado-trastuzumab emtansine; afatinib dimaleate; afimoxifene; aldesleukin; alectinib; alemtuzumab; alpelisib; amifostine; anastrozole; apalutamide; aprepitant; arsenic trioxide; asparaginase *erwinia chrysanthemi*; atezolizumab; avapritinib; avelumab; axicabtagene ciloleucel; axitinib; azacitidine; AZD9833 (AstraZeneca); AZD9496 (AstraZeneca); bazedoxifene; belinostat; bendamustine hydrochloride; bevacizumab; bexarotene; bicalutamide; binimetinib; bleomycin sulfate; blinatumomab; bortezomib; bosutinib; brentuximab vedotin; brigatinib; cabazitaxel; cabozantinib-s-malate; calaspargase pegol-mknl; capecitabine; caplacizumab-yhdp; capmatinib hydrochloride; carboplatin; carfilzomib; carmustine; cemiplimab-rwlc; ceritinib; cetuximab; chlorambucil; cisplatin; cladribine; clofarabine; cobimetinib; copanlisib hydrochloride; crizotinib; cyclophosphamide; cytarabine; D-0502 (Inventisbio); dabrafenib mesylate; dacarbazine; dacomitinib; dactinomycin; daratumumab; daratumumab and hyaluronidase-fihj; darbepoetin alfa; darolutamide; dasatinib; daunorubicin hydrochloride; decitabine; defibrotide sodium; degarelix; denileukin diftotox; denosumab; dexamethasone; dexrazoxane hydrochloride; dinutuximab; docetaxel; doxorubicin hydrochloride; durvalumab; duvelisib; elacestrant; elotuzumab; eltrombopag olamine; emapalumab-lzsg; enasidenib mesylate; encorafenib; enfortumab vedotin-ejfv; entrectinib; enzalutamide; epirubicin hydrochloride; epoetin alfa;

erdafitinib; eribulin mesylate; erlotinib hydrochloride; etoposide; etoposide phosphate; everolimus; exemestane; fam-trastuzumab deruxtecan-nxki; fedratinib hydrochloride; filgrastim; fludarabine phosphate; fluorouracil; flutamide; fostamatinib disodium; fulvestrant; gefitinib; gemcitabine hydrochloride; gemtuzumab ozogamicin; gilteritinib
5 fumarate; glasdegib maleate; glucarpidase; goserelin acetate; granisetron; granisetron hydrochloride; hydroxyurea; ibritumomab tiuxetan; ibrutinib; idarubicin hydrochloride; idelalisib; ifosfamide; imatinib mesylate; imiquimod; inotuzumab ozogamicin; interferon alfa-2b recombinant; iobenguane I-131; ipatasertib; ipilimumab; irinotecan hydrochloride; isatuximab-irfc; ivosidenib; ixabepilone; ixazomib citrate; lanreotide acetate; lapatinib
10 ditosylate; larotrectinib sulfate; lasofoxifene; lenalidomide; lenvatinib mesylate; letrozole; leucovorin calcium; leuprolide acetate; lomustine; lorlatinib; LSZ102 (Novartis); lurbinectedin; LY3484356 (Lilly); megestrol acetate; melphalan; melphalan hydrochloride; mercaptopurine; methotrexate; midostaurin; mitomycin; mitoxantrone hydrochloride; mogamulizumab-kpkc; moxetumomab pasudotox-tdfk; necitumumab; nelarabine;
15 neratinib maleate; nilotinib; nilutamide; niraparib tosylate monohydrate; nivolumab; obinutuzumab; ofatumumab; olaparib; omacetaxine mepesuccinate; ondansetron hydrochloride; osimertinib mesylate; oxaliplatin; paclitaxel; paclitaxel albumin-stabilized nanoparticle formulation; palifermin; palonosetron hydrochloride; pamidronate disodium; panitumumab; panobinostat; pazopanib hydrochloride; pegaspargase; pegfilgrastim;
20 peginterferon alfa-2b; pembrolizumab; pemetrexed disodium; pemigatinib; pertuzumab; pexidartinib hydrochloride; plerixafor; polatuzumab vedotin-piiq; pomalidomide; ponatinib hydrochloride; pralatrexate; prednisone; procarbazine hydrochloride; propranolol hydrochloride; radium 223 dichloride; raloxifene hydrochloride; ramucirumab; rasburicase; ravulizumab-cwvz; recombinant interferon alfa-2b; regorafenib; RG6171
25 (Roche); rintodestrant; ripretinib; rituximab; rolapitant hydrochloride; romidepsin; romiplostim; rucaparib camsylate; ruxolitinib phosphate; sacituzumab govitecan-hziy; SAR439859 (Sanofi); selinexor; selpercatinib; selumetinib sulfate; SHR9549 (Jiansu Hengrui Medicine); siltuximab; sipuleucel-t; sonidegib; sorafenib tosylate; tagraxofusp-erzs; talazoparib tosylate; talimogene laherparepvec; tamoxifen citrate; tazemetostat
30 hydrobromide; temozolomide; temsirolimus; thalidomide; thioguanine; thiotepa; tisagenlecleucel; tocilizumab; topotecan hydrochloride; toremifene; trabectedin; trametinib; trastuzumab; trastuzumab and hyaluronidase-oysk; trifluridine and tipiracil hydrochloride; tucatinib; uridine triacetate; valrubicin; vandetanib; vemurafenib; venetoclax; vinblastine sulfate; vincristine sulfate; vinorelbine tartrate; vismodegib;
35 vorinostat; zanubrutinib; ziv-aflibercept; ZN-c5 (Zentalis); and zoledronic acid; or free

base, pharmaceutically acceptable salt, or solvate forms of the foregoing; or combinations thereof.

Pharmaceutical Compositions, Medicaments and Kits

The present invention further provides pharmaceutical compositions, medicaments and kits comprising a compound of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

R¹ is -L-(5-6 membered heteroaryl) or -L-(phenyl), where said 5-6 membered heteroaryl or phenyl is optionally substituted by one to three R³;

R² is C₁-C₆ alkyl or C₃-C₇ cycloalkyl, where said C₃-C₇ cycloalkyl is optionally substituted by C₁-C₄ alkyl;

L is a bond or a methylene; and

each R³ is independently C₁-C₄ alkyl, C₁-C₄ alkoxy or SO₂-C₁-C₄ alkyl, where each C₁-C₄ alkyl is optionally substituted by F, OH or C₁-C₄ alkoxy.

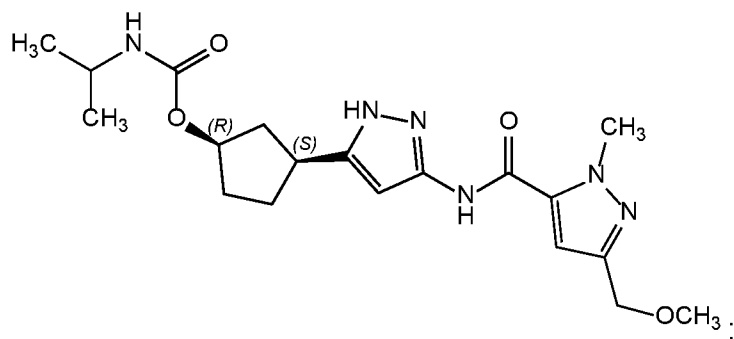
In another embodiment, the invention provides a pharmaceutical composition comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof, a CDK2 inhibitor or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or excipient. In some embodiments of this aspect, the pharmaceutical composition further comprises an additional anti-cancer agent.

In another embodiment, the invention provides a first pharmaceutical composition comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or excipient, and a second pharmaceutical composition comprising a CDC25A inhibitor or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or excipient, wherein the first and second pharmaceutical compositions are administered simultaneously, sequentially or separately. Some embodiments of this aspect further comprise a third pharmaceutical composition comprising an additional anti-cancer agent, and a pharmaceutically acceptable carrier or excipient, wherein the first, second and third pharmaceutical compositions are administered simultaneously, sequentially or separately.

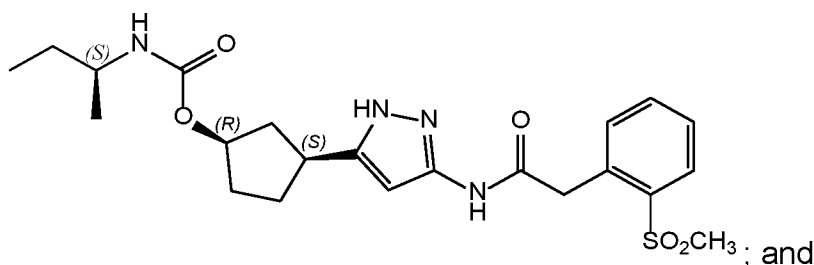
In another embodiment, the invention provides a combination comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof, and a CDC25A inhibitor or a pharmaceutically acceptable salt thereof, for use in the manufacture of a medicament for treating cancer in a subject. In another aspect, the invention provides use of a combination comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof, and a CDC25A inhibitor or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treating cancer in a subject. In some embodiments of these aspects, the combination further comprises an additional anti-cancer agent (e.g., an endocrine therapeutic agent) for use in the manufacture of a medicament.

In another embodiment, the invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof for use in the manufacture of a medicament for treating cancer, wherein the medicament is adapted for use in combination with a CDC25A inhibitor or a pharmaceutically acceptable salt thereof. In another embodiment, the invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof for use in the manufacture of a medicament for treating cancer, wherein the medicament is adapted for use in combination with a CDC25A inhibitor or a pharmaceutically acceptable salt thereof, and an additional anti-cancer agent. In another embodiment, the invention provides use of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treating cancer, wherein the medicament is adapted for use in combination with a CDC25A inhibitor or a pharmaceutically acceptable salt thereof. In another aspect, the invention provides use of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treating cancer, wherein the medicament is adapted for use in combination with a CDC25A inhibitor or a pharmaceutically acceptable salt thereof, and an additional anti-cancer agent.

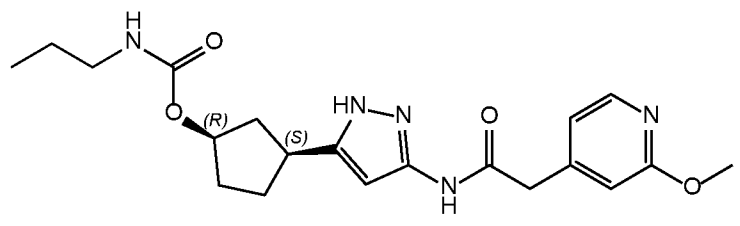
In some embodiments of each of the pharmaceutical compositions and medicaments described herein, the compound of Formula (I) is selected from the group consisting of (1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A), having the structure:



(1*R*,3*S*)-3-[3-({[2-(methylsulfonyl)phenyl]acetyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate (COMPOUND B), having the structure:

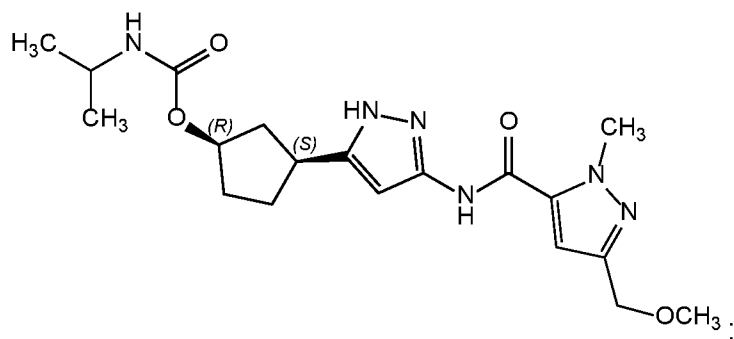


5 (1*R*,3*S*)-3-[3-({[2-(methoxypyridin-4-yl)acetyl]amino}-1*H*-pyrazol-5-yl)cyclopentyl propylcarbamate (COMPOUND C), having the structure:

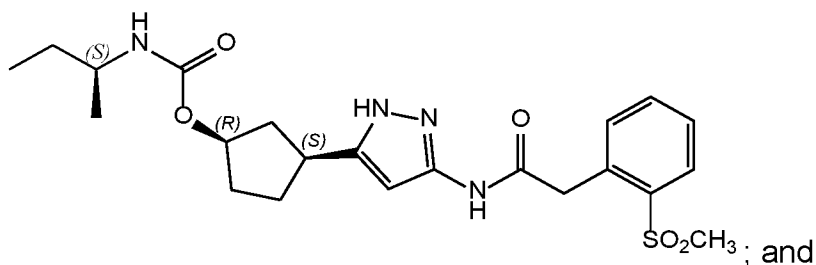


or a pharmaceutically acceptable salt thereof.

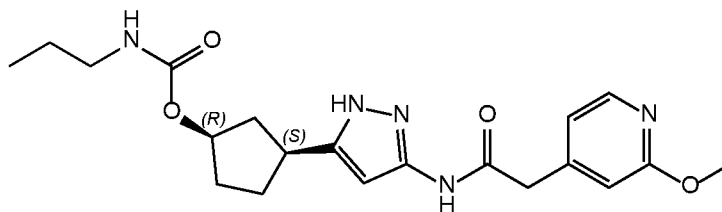
10 In some embodiments of each of the pharmaceutical compositions and medicaments described herein, the compound of Formula (I) is selected from the group consisting of (1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A), having the structure:



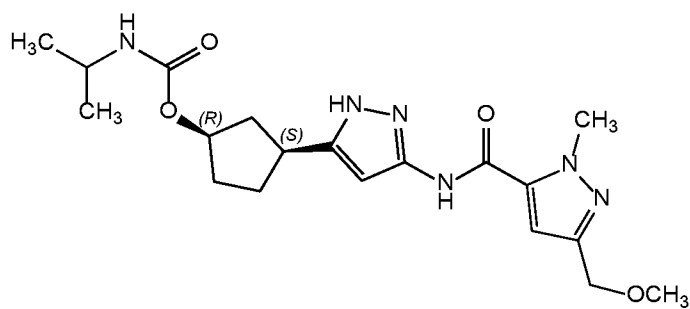
15 (1*R*,3*S*)-3-[3-({[2-(methylsulfonyl)phenyl]acetyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate (COMPOUND B), having the structure:



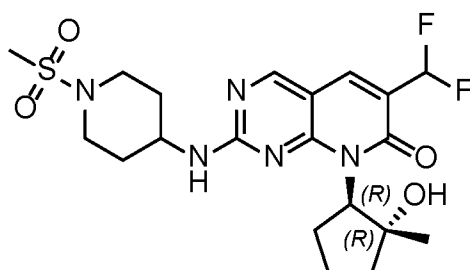
(1*R*,3*S*)-3-(3-((2-methoxypyridin-4-yl)acetyl)amino)-1*H*-pyrazol-5-yl)cyclopentyl propylcarbamate (COMPOUND C), having the structure:



- 5 In some embodiments of each of the pharmaceutical compositions and medicaments described herein, the CDK2 inhibitor is (1*R*,3*S*)-3-[3-((3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl)carbonyl)amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A), having the structure:



- 10 In some embodiments of each of the pharmaceutical compositions and medicaments described herein, the CDK2 inhibitor is 6-(difluoromethyl)-8-((1*R*,2*R*)-2-hydroxy-2-methylcyclopentyl)-2-(1-(methylsulfonyl)-piperidin-4-ylamino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF-06873600), having the structure:



- 15 or a pharmaceutically acceptable salt thereof.
- In another embodiment, the invention provides a kit comprising a first container, a second container and a package insert, wherein the first container comprises at least

one dose of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, as further described herein; the second container comprises at least one dose of a CDC25A inhibitor or a pharmaceutically acceptable salt thereof; and the package insert comprises instructions for treating cancer in a subject using the medicaments. In another embodiment, the invention provides a kit comprising a first container, a second container, a third container, and a package insert, wherein the first container comprises at least one dose of a compound of Formula (I) or a pharmaceutically acceptable salt thereof; the second container comprises at least one dose of a CDC25A inhibitor or a pharmaceutically acceptable salt thereof; the third container comprises at least one dose of an additional anti-cancer agent; and the package insert comprises instructions for treating cancer in a subject using the medicaments.

In some such embodiments, the kit of the present invention may include one or more containers comprising an agent or combination of agents (e.g., a CDK2 inhibitor and a CDC25A inhibitor), one or more anti-cancer agents, and/or one or more chemotherapeutic agents.

In some embodiments, the kits further include instructions for use in accordance with the methods, combinations and uses of the present invention. In some embodiments, these instructions comprise a description of administration of the agent to treat or diagnose, e.g., a cancer, according to any of the methods of the present invention. In some embodiments, the instructions comprise a description of how to detect a certain class of cancer, for example in an individual, in a tissue sample, or in a cell.

The kit may further comprise a description of selecting an individual suitable for treatment based on identifying whether that subject has a specific type of cancer. The instructions generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the present invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable. The label or package insert indicates that the composition is used for treating, e.g., a class of cancer, in a subject. Instructions may be provided for practicing any of the methods described herein.

The kits of the present invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or

an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (e.g., the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). In certain embodiments, at least one agent, or combination of agents in the composition is a CDK2 inhibitor or CDC25A inhibitor. The container may further comprise a second and/or additional pharmaceutically active agent. Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container.

All publications and patent applications cited in the specification are herein incorporated by reference in their entirety. It will be apparent to those of ordinary skill in the art that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); aa, amino acid(s); nt, nucleotide(s); s or sec, second(s); min, minute(s); h or hr, hour(s); mer, oligo length; false discovery rate, FDR; standard deviation, SD; onset or baseline, T0; after 24 h, T1; 2 days, T2; 4 days, T4; 7 days, T7; 11 days, T11; 14 days, T14; 18 days, T18; and the like.

Example 1: CRISPR Screening Identified CDC25A as a Synthetic Lethal Hit for CDK2 Inhibition

Overview

To discover genes synthetic lethal with CDK2 inhibition, CRISPR screens were performed to identify sensitizers to CDK2 and CDK2/4/6 inhibition in

multiple cancer cell models with either focused cell cycle sgRNA library or genome-wide libraries (PINCER and TKOV3) as described below. CDC25A was identified as a strong synthetic lethal hit for both CDK2-selective and CDK2/4/6 inhibition in small cell lung cancer cell, pancreatic cancer cell and ER+ breast cancer cell models.

5 Materials and Methods

1. PINCER CRISPR library Construction

Six sgRNA protospacer sequences were selected for every human and mouse gene in the genome. Every gene in the genome was assigned to a screening priority tier, with tier 1 indicating the highest priority and tier 3 the lowest priority. Tier 1 included 8023 genes that were considered to be druggable (ChEMBL, (Finan *et al.*, The druggable genome and support for target identification and validation in drug development, *Sci Transl Med*, 2017, 9, eaag1166), kinases (Uniprot, HGNC, or CSHL), epigenetic regulators, proteases), or de-ubiquitinating enzymes, ion channels, solute carriers, known oncogenes and tumor suppressor genes (Foundation One or Broad TARGET), DNA damage response genes, and nuclear hormone receptors. Tier 2 included 2971 genes that were annotated to be exported or cell surface proteins, GPCRs, Olfactory receptors and transmembrane or exported. Tier 3 included the remainder of the genome, totaling 8277 genes. A priority screening library was constructed by including six sgRNAs per gene for genes in tiers 1 and 2.

20 2. Cloning

To construct sgRNA expression vectors, 83nt DNA sequences (SEQ ID NO:1) were assembled by flanking 20nt guide protospacers (SEQ ID Nos 2 to 29) with Esp3I restriction sites and polymerase chain reaction (PCR) primer template (Table 1). The “n” could be any of the 20 nucleotides set forth in SEQ ID Nos 2 to 29.

25 Table 1

SEQ ID NO	Description	SEQUENCE
1	83nt DNA sequences	GAGGCTCAACCTGACGATACCGTCTCTCACCGnGTTT GGAGACGCTGTCAGTAGTGGATGCAGG
2	TKOV3	GTAAAGATCTCTTCACACAG
3	TKOV3	GATGTAGTCCAGCTCTGAAG
4	TKOV3	CAAGGAACTAACTTACAGGT
5	TKOV3	GCCAAATAGCGCCTTCACGA
6	Pincer	ATACGAGGGAGGCCACATCA
7	Pincer	CGATACCCATATGAATACGA
8	Pincer	GAGCTGTATGTCCTGAAGGG
9	Pincer	GGGTGCAGTGAACCTTGCACA

10	Pincer	GTGAGAGAGAGAGATCGCCT
11	Pincer	TACCCATATGAATACGAGGG
12	Cell cycle	ACTCCTTGTATCCCCCCTTC
13	Cell cycle	CCCTTCAGGACATACAGCTC
14	Cell cycle	GTTTGGGGTATTTCATTACCC
15	Cell cycle	ATGACACGCTTGCCATCAGT
16	Cell cycle	GGCCTCCCTCGTATTCATAT
17	Cell cycle	TGATAACAACTCTTTAATG
18	Cell cycle	GA CTCCTCTTGAGATCGTTC
19	Cell cycle	CTTCAACACTGACCGAGTGC
20	Cell Cycle	ACCGAGTGCTGGAGCTACAC
21	Cell cycle	CAGAGGCTTGCCATGCACGA
22	Cell cycle	CCAGTAAGACCTGTATCTCG
23	Cell cycle	GGCTTCGTGGACCTTCTCGA
24	Cell cycle	CGATACCCATATGAATACGA
25	Cell cycle	TACCCATATGAATACGAGGG
26	Cell cycle	TGGCCTCCCTCGTATTCATA
27	Cell cycle	TGTCCTGAAGGGGGGATACA
28	Cell cycle	ACTCCTTGTATCCCCCCTTC
29	Cell cycle	CCCTTCAGGACATACAGCTC

Lyophilized and cleaved 83-mer (83nt DNA sequences) were used (5000 oligo “SureGuide Unamplified Custom CRISPR Library”, Part Number: G7555B; Agilent), re-suspended in water, and amplified by PCR. Next, guides were excised by Esp3I at 21°C for 3 hours and ligated into a modified version of Tarumoto et al.’s LRG2.1 vector, in which green fluorescence protein was replaced by puromycin resistance (pac) using BamHI+BsrGI (Tarumoto, *et al.*, LKB1, Salt-Inducible Kinases, and MEF2C Are Linked Dependencies in Acute Myeloid Leukemia, *Mol Cell*, 2018, 69, 1017-1027 e1016; Grevet, *et al.*, Domain-focused CRISPR screen identifies HRI as a fetal hemoglobin regulator in human erythroid cells, *Science*, 2018, 361, 285-290).

Finally, vectors were electroporated into four replicates of STBL4 cells and pooled. A constitutive Cas9 expression vector was constructed as follows. First, an empty lentiviral vector with ampicillin resistance (pLV7-Empty) was constructed by restricting pLenti7.3/V5-DEST (Thermo Fisher Scientific, #V53406) with ClaI and Acc65I, then re-ligating by duplexed oligonucleotides, pLV7-Empty ligation oligonucleotides (FWD) (SEQ ID NO: 30) and (pLV7-Empty ligation oligonucleotides (REV) (SEQ ID NO: 31). The EF-1 Alpha short (EFS) promoter was added (pLV7-EFS), by PCR amplifying EFS, restricting pLV7-Empty with XbaI and BamHI, and ligation. Next, the reverse-complement DNA sequence encoding human-optimized 3X FLAG-tagged Cas9 was extracted from its source publication (Ran, *et al.*, Genome engineering using the CRISPR-Cas9 system, *Nat Protoc.* 2013, 8, 2281-2308). Cas9 DNA was synthesized in three fragments with

flanking BamHI and NheI restriction sites (ATUM, DNA2.0), 2A self-cleaving peptide sequence (P2A) oligonucleotides were synthesized, P2A oligonucleotides (FWD) (SEQ ID No:32) and P2A oligonucleotides (REV) (SEQ ID NO: 33), and the hygromycin resistance gene (hph, Hygro) was synthesized with flanking Esp3I and MluI restriction sites (ATUM, DNA2.0). Finally, Cas9, P2A, and Hygro were ligated together, pLV7-EFS
5 was restricted by BamHI and MluI, and Cas9-P2A-Hygro was inserted by ligation to generate the final vector (pLV7-EFS-Cas9-P2A-Hygro) (SEQ ID NO: 34).

3. Tissue culture

Cell lines H1048, H1876, H82, H211, MCF7, Hs766T and SU8686 were purchased
10 from the American Type Culture Collection (ATCC) and were maintained in conditions suggested by ATCC. Cell lines stably expressing Cas9 were generated by lentiviral transduction at a low multiplicity of infection to introduce a single Cas9 copy per cell and selected using hygromycin at 250 µg/mL (H1048, H1876, H82, H211, MCF7 and SU8686) or 400 µg/mL (Hs766T and HCC1428). Cells expressing sgRNA were selected
15 using puromycin at 1 to 2 µg/mL.

4. Lentiviral transduction

Lentivirus was produced by transfecting cells with Sigma Lentiviral packaging mix (Sigma #SHP 001). Briefly, 3 µg of plasmid DNA, 3 µg of Sigma-Lentiviral packaging mix and 36 µl of Lipofectamine 2000 (Invitrogen #1348310) were mixed, incubated and added
20 to the 10 cm plate HEK293T cells. Media was replaced 6 to 8 hr post transfection. Virus was collected 48 and 72 hr post transfection and pooled.

Cell lines were infected with virus-containing plain medium for 24 hr. Medium was then replaced with puromycin containing medium to select for transduced cells, and incubated for 3 to 7 days. Optimal infection conditions were determined for each batch
25 of virus prep in each cell line. After antibiotic selection, live cells were counted. Volumes of virus that yielded ~10 to 30% infection efficiency were used for screening (10 to 30% of live cells remained relative to no antibiotic selection control).

5. Pooled Screening and Sequencing for sgRNA Abundance

For all screens, cells with stable Cas9 expression were infected in three biological
30 replicates per cell line with lentiviral sgRNA pools at a representation of 230 to 1000 cells per sgRNA at a MOI of 0.10 to 0.30. Cells were selected in the presence of puromycin, and a sample was collected 3 to 7 days post-selection as a reference representation of the pooled sgRNA library. Pooled CRISPR sgRNA libraries used in the individual cell

lines are as follows: Cell Cycle guide library (obtained from collaboration with CSHL) in H1048, H1876 and H82; PINCER guide library (described above) in HCC1428 and SU8686; and TKOv3 guide library in MCF7 and Hs766T cell lines.

Each cell line was simultaneously screened with DMSO control and different CDK inhibitors. Concentrations of inhibitors tested against each cell line were those that yielded ~25% for H1048, H1876 and H82, while ~50% inhibition of proliferation for HCC1428, MCF7, SU8686, and Hs766T in 6-day proliferation assays. Cells were propagated for a total of 14 days (in H1048, H82, H1876, SU8686 and Hs766T cells) or 21 days (in HCC1428 and MCF7 cells) maintained at each passage. For HCC1428, MCF7, SU8686, and Hs766T, an average representation of 400 cells per sgRNA was maintained at each passage. For the H1048, H82, H1876 an average representation of > 1000 cells per sgRNA was maintained at each passage. Cells were harvested for genomic DNA extraction (DNeasy Blood and Tissue kit, Qiagen Cat#69506). sgRNA inserts were PCR amplified and purified by QIAquick PCR Purification Kit (Qiagen, Cat#28106) (SEQ ID Nos 35 and 36). The PCR products from the genomic DNA were then further amplified using secondary PCR primers harboring Illumina TruSeq adapters i5 and i7 barcodes (SEQ ID Nos 37 to 46). The 300 bp PCR product were purified by gel extraction (Qiagen, Cat#28706). The resulting fragments were sequenced on a MiSeqTM (Illumina) with standard primers for dual indexing. The sequencing recipe we used included 33 “dark cycles” of base incorporation without imaging, followed by 21 light cycles with two indices. A simplified workflow is shown in FIG. 1.

6. Screen analysis

Sequence reads were aligned to sgRNAs with zero mismatches by Bowtie and Oculus (Langmead, *et. al.*, Ultrafast and memory-efficient alignment of short DNA sequences to the human genome, *Genome Biol.* 2009,10, R25; Veeneman, *et. al.*, Oculus: faster sequence alignment by streaming read compression, *BMC Bioinformatics* 2012,13, 297).

sgRNAs with fewer than 10 counts in any T0 sample were discarded. Significant gene-level depletion and enrichment in straight-lethal and synthetic-lethal context was assessed by MAGeCK-VISPR's maximum likelihood estimation method (Li, W., *et. al.*, Quality control, modeling, and visualization of CRISPR screens with MAGeCK-VISPR,

Genome Biol, 2015, 16, 281). In all cases, treatment was compared to DMSO control with early sgRNA representation included as baseline.

Results

In three RB- SCLC cell models, sensitizer CRISPR screens were performed with focused cell cycle sgRNA library (targeting 360 genes). A few synthetic lethal hits were selected based on deconvoluting (Synthetic Lethal Beta Value) and non-deconvoluting (time factor) FDR < 0.1, beta score < 0 in each cell lines. As shown in FIG. 2, there is only 1 common synthetic lethal hit shared by 3 cell lines with CDK2i (FIG. 2A) and CDK2/4/6i (FIG. 2B). CDC25A was identified as a common synthetic lethal hit with both compounds ((1*R*,3*S*)-3-[3-({2-(methylsulfonyl)phenyl}acetyl)amino]-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate (COMPOUND B) and 6-(difluoromethyl)-8-((1*R*,2*R*)-2-hydroxy-2-methylcyclopentyl)-2-(1-(methylsulfonyl)piperidin-4-ylamino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF-06873600).

CDC25A was also synthetic lethal with both CDK2 and CDK2/4/6 inhibition in pancreatic (SU8686) and ER+ breast (HCC1428 and MCF7) cancer cell line models based on the same hit selection criteria. Results from all the CRISPR screens performed with CDK2i and CDK2/4/6i are summarized in Table 2 and Table 3 respectively.

Table 2

Cell lines	Control Beta Value	CDK2i Beta Value	CDK2i Synthetic Lethal Beta Value	CDK2i Synthetic Lethal FDR	CRISPR sgRNAs library
H1048	-0.07	-1.44	-1.37	0.00	Cell cycle
H82	-0.66	-1.27	-0.61	0.00	Cell cycle
H1876	-0.21	-0.54	-0.32	0.00	Cell cycle
SU8686	-0.14	-1.55	-1.41	0.00	PINCER
Hs766T	0.31	-0.02	-0.33	0.38	TKOv3
HCC1428	-0.26	-1.00	-0.73	0.00	PINCER
MCF7	0.46	-0.60	-1.06	0.00	TKOv3

Table 3

Cell lines	Control Beta Value	CDK2/4/6i Beta Value	CDK2/4/6i Synthetic Lethal Beta Value	CDK2/4/6i Synthetic Lethal FDR	CRISPR sgRNAs library
H1048	-0.07	-1.57	-1.5	0.00	Cell cycle
H82	-0.66	-1.29	-0.63	0.00	Cell cycle
H1876	-0.21	-0.62	-0.41	0.00	Cell cycle
SU8686	-0.15	-0.98	-0.83	0.00	PINCER

Hs766T	0.31	-0.08	-0.39	0.18	TKOv3
HCC1428	-0.26	-0.84	-0.58	0.00	PINCER
MCF7	0.46	-0.33	-0.79	0.03	TKOv3

Conclusions

Multiple CRISPR screens identified CDC25A as a strong synthetic lethal hit for both CDK2-selective and CDK2/4/6 inhibition in small cell lung cancer models, pancreatic cancer models and ER+ breast cancer models.

5 **EXAMPLE 2: Validation of CDC25A as a Synthetic Lethal Hit for CDK2 Inhibition by CRISPR Knockout in Small Cell Lung Cancer (SCLC) Cell Models**

Overview

To validate CRISPR screens results, green fluorescent protein (GFP) proliferation competition experiments were performed with CRISPR-sgRNAs in both RB- and RB+ SCLC cancer cell models. CDC25A knockout with 4 validated independent CRISPR-sgRNAs strongly synergized with IC25 concentrations of CDK2 and CDK2/4/6 inhibitors.

Materials and Methods

sgRNA competition assays (shown in FIG. 3A) were performed using the U6-sgRNA-EFS-GFP plasmids (which express the sgRNA and GFP from constitutive promoters (Addgene Plasmid #108098)). These plasmids were used to generate lentivirus by transfecting HEK293T cells as described in Example 1. After lentiviral infection in cell lines stably expressing Cas9, GFP percentages in each cell lines were tracked by LSRII flow cytometry over time. Gating was performed on live cells using forward and side scatter, prior to measuring of GFP positivity. The sgRNA-induced proliferation arrest was monitored by GFP-negative cells outcompeting GFP-positive cells, which was represented by the percentages of GFP-positive cells at individual time points normalized to that at T0 time point (day 3 after lentiviral sgRNAs infection).

Vehicle control or CDK inhibitors were added at T0 time point and refreshed every 3-4 days. The concentrations of CDK inhibitors used were around IC25 for each cell line, except for that palbociclib used in H1048 and H1876 were 150 nM because these 2 RB-cell lines are insensitive to palbociclib.

Results

The sgRNA targeting mouse Rosa26 locus was determined as a negative control, and the sgRNA targeting human RPA3 gene was determined as a positive control. For

CDC25A knockout, we used 4 independent sgRNAs targeting 4 different regions at CDC25A exons. The sgRNA-induced proliferation arrest was tracked over time by monitoring percentages of GFP expressing cells. Relative cell viability was calculated as %GFP+ cells at individual time point normalized to %GFP+ cells at T0 in each treatment arm. The variability across different CDC25A sgRNAs was represented by the error bar (SD). As shown in FIG.s 3B-D, in both RB- (H1048 and H1876) and RB+ (H211) SCLC cell line models, CDK inhibitors treatments did not impact control sgRNAs and were grouped together with Vehicle control. However, CDC25A knockout inhibited cell proliferation much stronger when combined with either CDK2 inhibitor or CDK2/4/6 inhibitor. CDC25A knockout did not synergize to CDK4/6 inhibition by palbociclib, suggesting the synergistic effects with CDK2/4/6 inhibitor were mainly due to CDK2 inhibition.

Conclusions

CDC25A was validated as a synthetic lethal target with CDK2 inhibition by CRISPR knockout in SCLC cell models.

EXAMPLE 3: Validation of CDC25A as a Synthetic Lethal Hit for CDK2 Inhibition by shRNA Knockdown

Overview

To further validate CDC25A as a synthetic lethal target with CDK2 inhibition, doxycycline (DOX) inducible CDC25A knockdown cell lines were generated in H82 cells. CDC25A knockdown with 2 validated independent shRNAs synergized with both CDK2 and CDK2/4/6 inhibitors. Validation experiments by using another genetic tool is important, especially when there is a lack of CDC25A inhibitors with ideal selectivity and potency.

Materials and Methods

1. Cell line generation:

TET-ON shERWOOD-UltramiR shRNA constructs (Transomics pZIP-TRE3GS backbone, map can be found at: https://www.snapgene.com/resources/plasmid-files/?set=viral_expression_and_packaging_vectors&plasmid=pZIP-TRE3G-ZsGreen-Puro) were used for DOX inducible shRNA expression in H82 cells. Hairpin sequences included are Non-Target (NT) (SEQ ID NO: 47), shCDC25A_67 (SEQ ID NO: 48) and shCDC25A_72 (SEQ ID NO: 49). Lentivirus was generated by transfecting HEK293T

cells with shRNA constructs using the same method described in Example 1. Infected H82 cells were selected under 1 µg/mL puromycin. Cells were treated with 1 µg/mL DOX to induce shRNA expression wherever indicated.

2. CyQUANT® Direct cell proliferation assay

5 After shRNA expression induced by DOX for 6 days, H82 cells were plated in 96-well flat-bottomed microplates at low density (4K cells/well). 1:3 serial diluted CDK inhibitors were added on top of the cells after seeding for at least 6 hours. DOX were maintained in culture at the same concentration. 7 days later, cell numbers were determined by CyQUANT® Direct Cell Proliferation Assay kit (Molecular Probes, Inc., Eugene, OR) following manufacturer's instructions. Dose-response curves were fitted to determine the IC50 values by GraphPad Prism. All error bars in FIG. 4 represent SD for 10 3 replicates.

Results

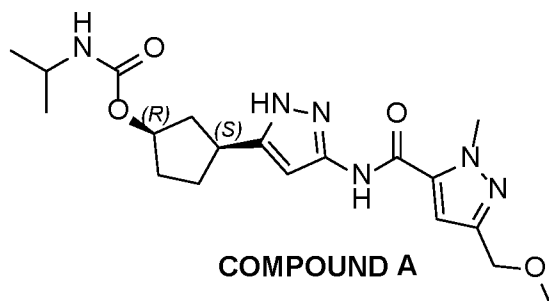
7-day proliferation assays were performed to determine the impact of CDC25A 15 knockdown on CDK inhibitors. CDC25A knockdown decreased IC50s of both CDK2 inhibitor (FIG. 4A) and CDK2/4/6 inhibitor (FIG. 4B) by 2 to 3 folds but did not change cell sensitivity to palbociclib (FIG. 4C), which is consistent with CRISPR knockout experiments shown in Example 2.

Conclusions

20 CDC25A was validated as a synthetic lethal target with CDK2 inhibition by shRNA knockdown in small cell lung cancer (SCLC) cell model H82 cells.

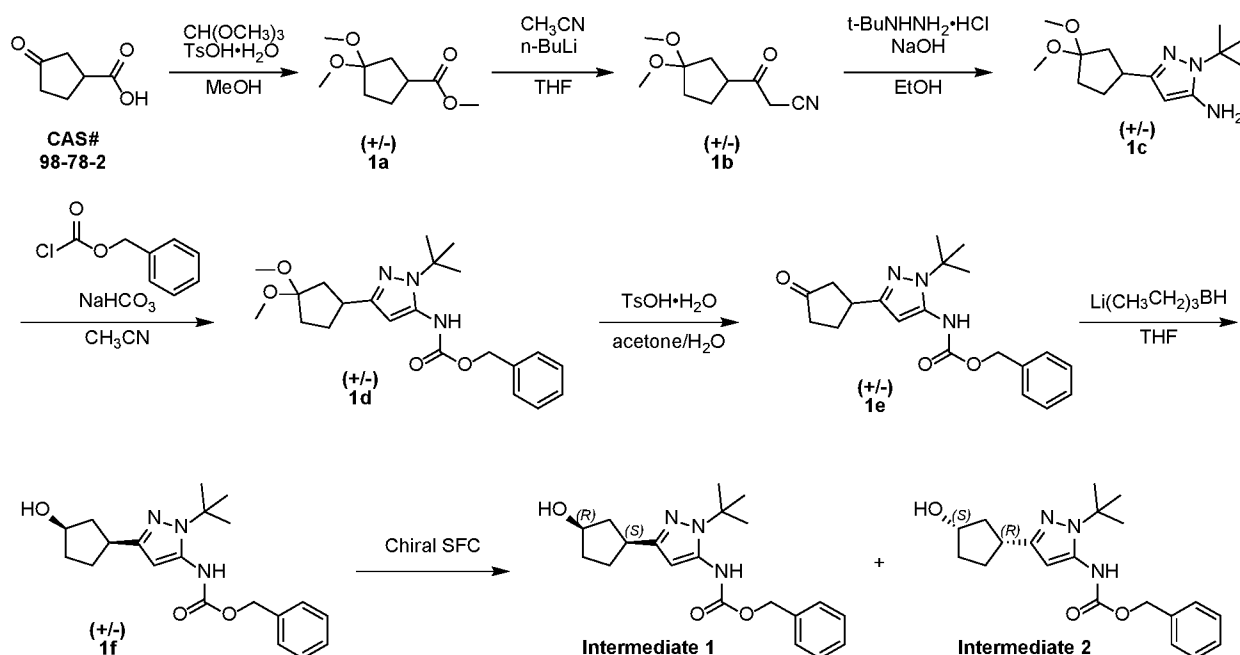
Example 4: Preparation of (1*R*,3*S*)-3-[3-([3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl)amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A)

25



COMPOUND A was prepared as described in Example 13 of U.S. 2020/0247784.

Preparation of Intermediate 1: benzyl {1-*tert*-butyl-3-[(1*S*,3*R*)-3-hydroxycyclopentyl]-1*H*-pyrazol-5-yl}carbamate; and Intermediate 2: benzyl {1-*tert*-butyl-3-[(1*R*,3*S*)-3-hydroxycyclopentyl]-1*H*-pyrazol-5-yl}carbamate.



Two parallel reactions, each containing a solution of (±)-3-oxocyclopentanecarboxylic acid (CAS#98-78-2, 900 g, 7.02 mol) in methanol (5 L) at 13 °C were each treated with trimethyl orthoformate (4.47 kg, 42.15 mol, 4.62 L) and 4-toluenesulfonic acid monohydrate (26.72 g, 140.5 mmol). The mixtures were stirred at 13 °C for 25 hours. Each batch was quenched separately with sat. aq NaHCO₃ (1 L), then the two batches were combined and concentrated under vacuum to remove most of the methanol. The residue was diluted with ethyl acetate (4 L), and the layers separated. The aqueous layer was further extracted with ethyl acetate (2 x 1L). The combined organic layers were washed with sat. aq NaCl (3 x 1L), dried over magnesium sulfate, filtered, and concentrated under vacuum to give (±)-methyl 3,3-dimethoxycyclopentanecarboxylate (1a, 2.5 kg, 13.28 mol, 94%) as a light yellow oil. ¹H NMR (400MHz, CHLOROFORM-*d*) δ = 3.67 (s, 3H), 3.20 (s, 3H), 3.19 (s, 3H), 2.94-2.82 (m, 1H), 2.16-2.00 (m, 2H), 1.99-1.76 (m, 4H).

A solution of *n*-butyllithium (3.44 L of a 2.5 M solution in hexanes, 8.6 mol) was added to a reactor containing THF (3 L) at -65 °C. Anhydrous acetonitrile (453 mL, 353 g, 8.61 mol) was added dropwise, slowly enough to maintain the internal temperature below -55 °C. The mixture was stirred for an additional 1 hour at -65 °C. A solution of (±)-methyl 3,3-dimethoxycyclopentanecarboxylate (1a, 810 g, 4.30 mol) in THF (1 L) was then added dropwise, slowly enough to maintain the internal temperature below -50 °C.

After stirring for an additional hour at -65 °C, the reaction was quenched with water (4 L), neutralized with aq HCl (1 M) to pH 7, and extracted with ethyl acetate (3 x 3L). The combined organic layers were washed with sat. aq NaCl (2 x 3L), dried over magnesium sulfate, filtered, and concentrated under vacuum to give crude (±)-3-(3,3-dimethoxycyclopentyl)-3-oxopropanenitrile (1b, 722 g, 3.66 mol, 85%) as a red oil, which was used without further purification.

Solid sodium hydroxide (131.4 g, 3.29 mol total) was added in portions to a suspension of *tert*-butylhydrazine hydrochloride (409.4 g, 3.29 mol) in ethanol (3 L) at 16-25 °C. Stirring was continued at 25 °C for 1 hour. A solution of crude (±)-3-(3,3-dimethoxycyclopentyl)-3-oxopropanenitrile (1b, 540 g, 2.74 mol) in ethanol was added at 25 °C, then the mixture was heated to 75 °C internal and stirred for 30 hours. The reaction was filtered, and the filtrate concentrated under vacuum to give crude product as a red oil. This product was combined with crude from three more identically-prepared batches (each starting with 540 g 1b; 2.16 kg, 10.96 mol total for the 4 batches), and purified by silica gel chromatography (eluting with 0-35% ethyl acetate in petroleum ether), affording (±)-1-*tert*-butyl-3-(3,3-dimethoxycyclopentyl)-1H-pyrazol-5-amine (1c, 1.60 kg, 5.98 mol, 54% yield) as a red oil. ¹H NMR (CHLOROFORM-*d*) δ = 5.41 (s, 1H), 3.50 (br. s., 2H), 3.22 (s, 3H), 3.20 (s, 3H), 3.13 (tt, J=7.9, 9.6 Hz, 1H), 2.25 (dd, J=8.0, 13.3 Hz, 1H), 2.09-2.00 (m, 1H), 1.99-1.91 (m, 1H), 1.83 (dd, J=10.8, 12.8 Hz, 2H), 1.78-1.68 (m, 1H), 1.60 (s, 9H).

Benzyl chloroformate (563.6 mL, 676.3 g, 3.96 mol) was added to a chilled (0-5 °C) solution of (±)-1-*tert*-butyl-3-(3,3-dimethoxycyclopentyl)-1H-pyrazol-5-amine (1c, 530 g, 1.98 mol) in acetonitrile (3.5 L). The mixture was stirred at 23 °C for 2 hours, and then solid sodium hydrogen carbonate (532.9 g, 6.34 mol) was added in portions. Stirring was continued at 23 °C for 26 hours. The resulting suspension was filtered and the filtrate concentrated under vacuum to give crude (±)-benzyl [1-*tert*-butyl-3-(3,3-dimethoxycyclopentyl)-1H-pyrazol-5-yl]carbamate (1d, 980 g, 1.98 mol max) as a red oil, which was used in the next step without further purification.

A solution of the crude (±)-benzyl [1-*tert*-butyl-3-(3,3-dimethoxycyclopentyl)-1H-pyrazol-5-yl]carbamate (1d, 980 g, 1.98 mol max) in acetone (2 L) and water (2 L) at 18 °C was treated with 4-toluenesulfonic acid monohydrate (48.75 g, 256.3 mmol). The mixture was heated to 60 °C internal for 20 hours. After concentration under vacuum to remove most of the acetone, the aqueous residue was extracted with dichloromethane (3 x 3 L). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated under vacuum to a crude red oil. This crude product was combined with

crude from two other identically-prepared batches (each derived from 1.98 mol 1c, 5.94 mol total for the 3 batches), and purified by silica gel chromatography (eluting with 0-50% ethyl acetate in petroleum ether) to give (±)-benzyl [1-*tert*-butyl-3-(3-oxocyclopentyl)-1*H*-pyrazol-5-yl]carbamate (1e, 1.6 kg) as a yellow solid. This solid was stirred in 10:1 petroleum ether/ethyl acetate (1.5 L) at 20 °C for 18 hours. The resulting suspension was filtered, the filter cake washed with petroleum ether (2 x 500 mL), and the solids dried under vacuum to give (±)-benzyl [1-*tert*-butyl-3-(3-oxocyclopentyl)-1*H*-pyrazol-5-yl]carbamate (1e, 1.4 kg, 3.9 mol, 66% combined for the three batches). ¹H NMR (DMSO-*d*₆) δ = 9.12 (br. s., 1H), 7.56-7.13 (m, 5H), 6.03 (s, 1H), 5.12 (s, 2H), 3.41-3.27 (m, 1H), 2.48-2.39 (m, 1H), 2.34-2.10 (m, 4H), 1.98-1.81 (m, 1H), 1.48 (s, 9H).

A solution of (±)-benzyl [1-*tert*-butyl-3-(3-oxocyclopentyl)-1*H*-pyrazol-5-yl]carbamate (1e, 320 g, 0.900 mol) in THF (1.5 L) was degassed under vacuum and purged with dry nitrogen (3 cycles), then cooled to -65 °C internal. A solution of lithium triethylborohydride (1.0 M in THF, 1.80 L, 1.80 mol) was added dropwise at a rate which maintained the internal temperature below -55 °C, then stirring was continued at -65 °C for 1.5 hours. The reaction mixture was quenched with sat. aq NaHCO₃ (1.5 L) at -40 to -30 °C. Hydrogen peroxide (30% aqueous, 700 g) was added to the mixture dropwise, while the internal temperature was maintained at -10 to 0 °C. The mixture was stirred at 10 °C for 1 hour, then extracted with ethyl acetate (3 x 2 L). The combined organic layers were washed with sat. aq Na₂SO₃ (2 x 1 L) and sat. aq NaCl (2 x 1 L). The organics were dried over magnesium sulfate, filtered, and concentrated under vacuum to a crude yellow oil. The crude product from this batch was combined with crude from three other, identically-prepared batches (each starting from 0.900 mol 1e, for a total of 3.60 mol) for purification. Before chromatography, the combined mixture showed ~3.3:1 *cis/trans* ratio by NMR. The combined crude product was purified twice by silica gel chromatography, eluting with 0-50% ethyl acetate in dichloromethane), affording (±)-*trans*-benzyl [1-*tert*-butyl-3-(3-hydroxycyclopentyl)-1*H*-pyrazol-5-yl]carbamate (1f, 960 g) as a light yellow solid, which was further purified by trituration, as described below.

A previous batch of 1f had been obtained from smaller-scale reactions, starting from a total of 120 g 1e (0.34 mol). The columned product from this batch was combined with the columned product from the batch above (which had been derived from 3.60 mol 1e, for a total of 3.94 mol 1e used for all the combined batches), suspended in 10:1 dichloromethane/methanol (1.5 L), and stirred at 20 °C for 16 hours. The suspension was filtered, and the filter cake washed with petroleum ether (2 x 500 mL). The solids were dried under vacuum to give clean (±)-*trans*-benzyl [1-*tert*-butyl-3-(3-

hydroxycyclopentyl)-1*H*-pyrazol-5-yl]carbamate (1f, 840 g, 2.35 mol, 60% total yield for all the combined batches) as a white solid. ¹H NMR (400MHz, DMSO-*d*₆) δ = 9.07 (br. s., 1H), 7.45-7.27 (m, 5H), 5.92 (s, 1H), 5.11 (s, 2H), 4.57 (d, J=4.5 Hz, 1H), 4.21-4.07 (m, 1H), 2.88 (quin, J=8.6 Hz, 1H), 2.24-2.13 (m, 1H), 1.92-1.78 (m, 1H), 1.78-1.62 (m, 2H), 1.61-1.53 (m, 1H), 1.47 (s, 9H), 1.52-1.43 (m, 1H). MS: 358 [M+H]⁺.

The enantiomers of (±)-*trans*-benzyl [1-*tert*-butyl-3-(3-hydroxycyclopentyl)-1*H*-pyrazol-5-yl]carbamate (1f, 700 g, 1.96 mol) were separated by chiral SFC.

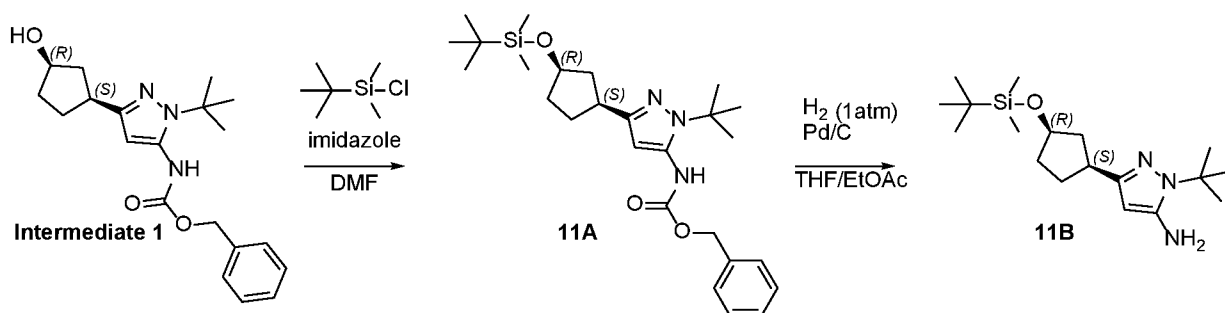
The product from the first-eluting enantiomer peak (310 g solid) was suspended in methanol/petroleum ether (1:10, 1 L) and stirred at 25 °C for 1 hour. The suspension was filtered, the filter pad washed with petroleum ether (2 x 500 mL), and the solids dried under vacuum to give benzyl {1-*tert*-butyl-3-[(1*S*,3*R*)-3-hydroxycyclopentyl]-1*H*-pyrazol-5-yl}carbamate (Intermediate 1, 255 g, 713 mmol, 36%, >99% ee) as a white solid. ¹H NMR (400MHz, DMSO-*d*₆) δ = 9.08 (br. s., 1H), 7.58-7.20 (m, 5H), 5.92 (s, 1H), 5.11 (s, 2H), 4.57 (d, J=4.4 Hz, 1H), 4.19-4.09 (m, 1H), 2.88 (quin, J=8.6 Hz, 1H), 2.24-2.13 (m, 1H), 1.91-1.79 (m, 1H), 1.79-1.61 (m, 2H), 1.61-1.53 (m, 1H), 1.47 (s, 9H), 1.52-1.44 (m, 1H). MS: 358 [M+H]⁺. Optical rotation [α]_D +3.76 (c 1.0, MeOH). Chiral purity: >99% ee, retention time 3.371 min. Chiral SFC analysis was performed on a ChiralPak AD-3 150 x 4.6 mm ID, 3 μm column heated to 40 °C, eluted with a mobile phase of CO₂ and a gradient of 0-40% methanol+0.05%DEA over 5.5 min, then held at 40% for 3 min; flowing at 2.5 mL/min.

The product from the second-eluting enantiomer peak (300 g solid) was suspended in methanol/petroleum ether (1:10, 1 L) and stirred at 25 °C for 1 hour. The suspension was filtered, the filter pad washed with petroleum ether (2 x 500 mL), and the solids dried under vacuum to give benzyl {1-*tert*-butyl-3-[(1*R*,3*S*)-3-hydroxycyclopentyl]-1*H*-pyrazol-5-yl}carbamate (Intermediate 2, 255 g, 713 mmol, 36%, 94% ee) as a white solid. ¹H NMR (400MHz, DMSO-*d*₆) δ = 9.08 (br. s., 1H), 7.55-7.19 (m, 5H), 5.92 (s, 1H), 5.11 (s, 2H), 4.57 (d, J=4.4 Hz, 1H), 4.23-4.07 (m, 1H), 2.88 (quin, J=8.7 Hz, 1H), 2.23-2.14 (m, 1H), 1.90-1.79 (m, 1H), 1.77-1.61 (m, 2H), 1.61-1.53 (m, 1H), 1.47 (s, 9H), 1.52-1.44 (m, 1H). MS: 358 [M+H]⁺. Optical rotation [α]_D -2.43 (c 1.0, MeOH). Chiral purity: 94% ee, retention time 3.608 min. Chiral SFC analysis was performed on a ChiralPak AD-3 150 x 4.6 mm ID, 3 μm column heated to 40 °C, eluted with a mobile phase of CO₂ and a gradient of 0-40% methanol+0.05%DEA over 5.5 min, then held at 40% for 3 min; flowing at 2.5 mL/min.

A sample of the second-eluting enantiomer from a previous batch with [α]_D -3.1 (c 1.1, MeOH) and 96% ee was crystalized from dichloroethane/pentane. A crystal structure

was obtained by small-molecule X-ray crystallography, which showed (1*R*,3*S*) geometry. The absolute stereochemistry of Intermediate 2 was thus assigned (1*R*,3*S*) based on its comparable optical rotation and order of elution in the analytical method. Intermediate 1, the enantiomer of Intermediate 2, was thus assigned (1*S*,3*R*) stereochemistry.

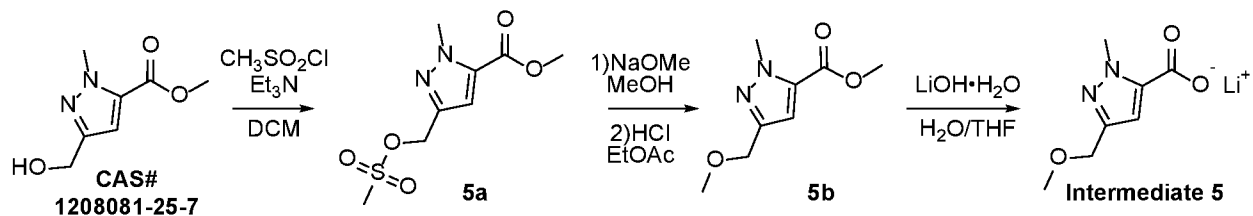
1. Preparation of Intermediate 11B: (1-*tert*-butyl-3-[(1*S*,3*R*)-3-{[*tert*-butyl(dimethyl)silyl]oxy}cyclopentyl]-1*H*-pyrazol-5-amine



Benzyl {1-*tert*-butyl-3-[(1*S*,3*R*)-3-hydroxycyclopentyl]-1*H*-pyrazol-5-yl}carbamate (Intermediate 1, 20 g, 56 mmol) and imidazole (5.71 g, 83.9 mmol) were dissolved in DMF (200 mL) with sonication. While the solution was at room temperature, *tert*-butyldimethylsilyl chloride (11.0 g, 72.7 mmol) was added in portions. After the addition was complete, the clear solution was stirred at 25 °C for 1 hour. The solvents were removed under vacuum and the residue partitioned between ethyl acetate (500 mL) and sat. aq NaCl (200 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated to give crude benzyl {1-*tert*-butyl-3-[(1*S*,3*R*)-3-{[*tert*-butyl(dimethyl)silyl]oxy}cyclopentyl]-1*H*-pyrazol-5-yl}carbamate (11A, 26 g, 99%) as a colorless oil. MS: 472 [M+H]⁺.

Crude benzyl {1-*tert*-butyl-3-[(1*S*,3*R*)-3-{[*tert*-butyl(dimethyl)silyl]oxy}cyclopentyl]-1*H*-pyrazol-5-yl}carbamate (11A, 26 g, 55 mmol) was dissolved in ethyl acetate (100 mL) and THF (100 mL). Added Pd/C (50% wet, 4 g), degassed the solution, and stirred at 25 °C under a hydrogen balloon for 2 hours. The mixture was then filtered, and the filtrate concentrated under vacuum to give crude 1-*tert*-butyl-3-[(1*S*,3*R*)-3-{[*tert*-butyl(dimethyl)silyl]oxy}cyclopentyl]-1*H*-pyrazol-5-amine (11B, 19 g, >99%) as a light yellow oil. MS: 338 [M+H]⁺.

2. Preparation of Intermediate 5: Lithium 3-(methoxymethyl)-1-methyl-1*H*-pyrazole-5-carboxylate.



A solution of methanesulfonyl chloride (11.32 g, 98.8 mmol) in dichloromethane (50 mL) was added dropwise to a cooled (0 °C) mixture of methyl 3-(hydroxymethyl)-1-methyl-1*H*-pyrazole-5-carboxylate (CAS# 1208081-25-7, 15.0 g, 88.1 mmol) and diisopropylethyl amine (14.8 g, 115 mmol) in dichloromethane (250 mL). The mixture was stirred at 0 °C for 45 minutes after the addition was complete. The reaction mixture was washed with sat. aq NH_4Cl , and the organic layer dried over sodium sulfate, filtered, and concentrated to give methyl 1-methyl-3-[(methanesulfonyl)oxy]methyl-1*H*-pyrazole-5-carboxylate (5a, 22.6 g, >99%) as a yellow oil, which was used without further purification.

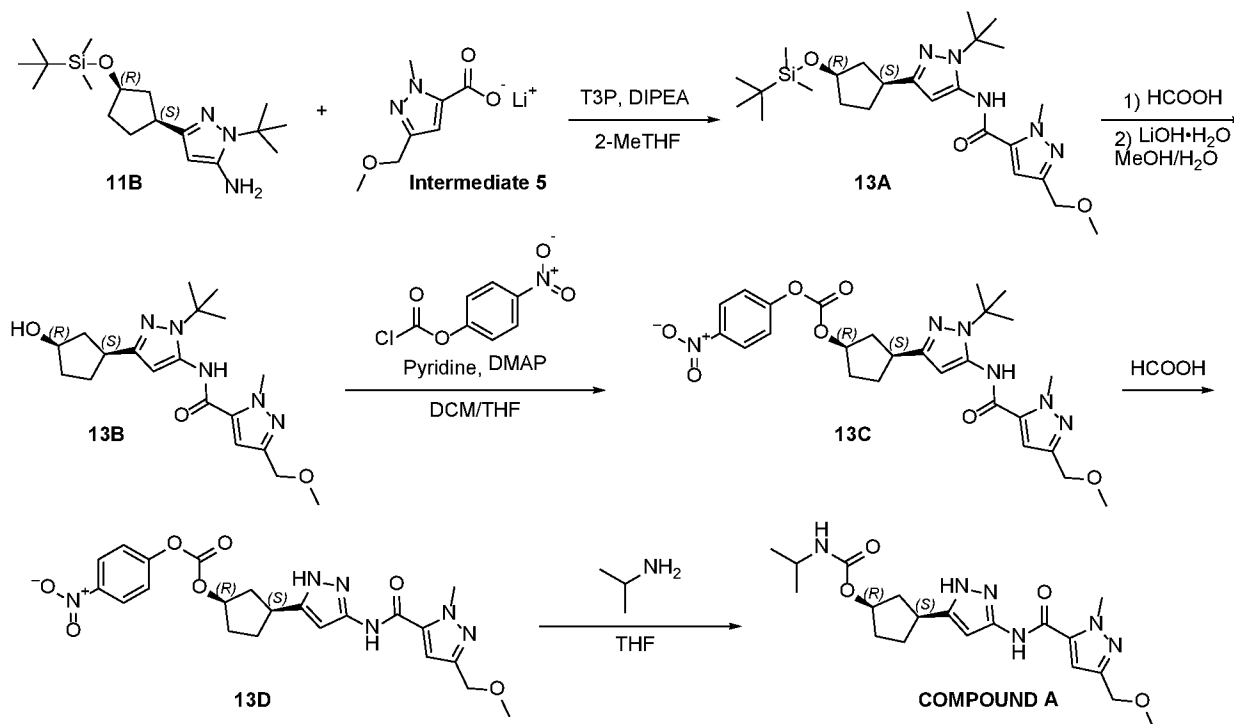
^1H NMR (400 MHz, CHCl_3 - d) δ = 6.98 (s, 1H), 5.26 (s, 2H), 4.20 (s, 3H), 3.91 (s, 3H), 3.03 (s, 3H).

A solution of methyl 1-methyl-3-[(methanesulfonyl)oxy]methyl-1*H*-pyrazole-5-carboxylate (5a, 22.6 g, 91.0 mmol) in methanol (200 mL) at room temperature was treated with solid sodium methoxide (9.84 g, 182 mmol) in small portions. The reaction was heated to 70 °C for 30 minutes. TLC suggested partial hydrolysis of the ester, so to re-esterify, the cloudy mixture was acidified with 4M HCl in ethyl acetate (40 mL, 160 mmol), and heating continued at 70 °C for 5 hours. The mixture was concentrated to dryness, leaving a white solid. This solid was extracted with ethyl acetate/petroleum ether (1/3, 3 x 200 mL). The combined extracts were concentrated to dryness, then the residual solid re-extracted with ethyl acetate/petroleum ether (1/3, 100 mL), dried over sodium sulfate, filtered, and concentrated to give methyl 3-(methoxymethyl)-1-methyl-1*H*-pyrazole-5-carboxylate (5b, 14.5 g, 86%, 80% pure by NMR) as a light yellow liquid which solidified on standing. Major component only: ^1H NMR (400 MHz, CHCl_3 - d) δ = 6.83 (s, 1H), 4.45 (s, 2H), 4.16 (s, 3H), 3.88 (s, 3H), 3.39 (s, 3H).

A solution of methyl 3-(methoxymethyl)-1-methyl-1*H*-pyrazole-5-carboxylate (5b, 14.5 g, 78.7 mmol) and lithium hydroxide monohydrate (3.47 g, 82.7 mmol) in THF (150 mL) and water (50 mL) was stirred at room temperature for 16 hours. The THF was removed under vacuum, and the residue dissolved in water (100 mL) and extracted with dichloromethane (3 x 30 mL). The organic layers were discarded. The aqueous layer was concentrated and dried under vacuum to give lithium 3-(methoxymethyl)-1-methyl-1*H*-pyrazole-5-carboxylate (Intermediate 5, 12.85 g, 92%) as a yellow solid. ^1H NMR

(400MHz, DMSO- d_6) δ = 6.37 (s, 1H), 4.24 (s, 2H), 4.01 (s, 3H), 3.20 (s, 3H). MS: 171 [M+H]⁺.

3. Preparation of COMPOUND A:



5 Propylphosphonic anhydride (T3P®, 50 wt% solution in EtOAc, 50.3 g, 79.1 mmol) was added to a room temperature (26 °C) solution of 1-tert-butyl-3-[(1S,3R)-3-[(tert-butyl(dimethyl)silyl]oxy]cyclopentyl]-1H-pyrazol-5-amine (11B, 8.90g, 26.4 mmol), lithium 3-(methoxymethyl)-1-methyl-1H-pyrazole-5-carboxylate (Intermediate 5, 5.83 g, 34.3 mmol), and diisopropylethyl amine (10.2 g, 79.1 mmol) in 2-methyltetrahydrofuran (100.0 mL). The resulting mixture was stirred at this temperature for 18 hours. After
10 concentrating the mixture to dryness, the residue was dissolved in dichloromethane (150 mL), and the solution washed sequentially with water (2 x 30 mL), sat. aq NaHCO₃ (2 x 30 mL) and sat. aq NaCl (30 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated to give crude N-{1-tert-butyl-3-[(1S,3R)-3-[(tert-butyl(dimethyl)silyl]oxy]cyclopentyl]-1H-pyrazol-5-yl}-3-(methoxymethyl)-1-methyl-1H-pyrazole-5-carboxamide (13A, 12.9 g, 100%) as an oil. MS: 490 [M+H]⁺.
15 The crude N-{1-tert-butyl-3-[(1S,3R)-3-[(tert-butyl(dimethyl)silyl]oxy]cyclopentyl]-1H-pyrazol-5-yl}-3-(methoxymethyl)-1-methyl-1H-pyrazole-5-carboxamide (13A, 12.9 g, 26.3 mmol) was dissolved in formic acid (80 mL) and stirred at room temperature (27 °C)
20 for 30 minutes. The mixture was concentrated to dryness, and the residue dissolved in methanol (80 mL). A solution of lithium hydroxide monohydrate (3.43 g, 81.8 mmol) in water (15 mL) was added, and the mixture stirred at room temperature (27 °C) for 1 hour.

The mixture was concentrated to dryness, and the residue was purified by silica gel chromatography (eluting with 0-80% ethyl acetate in petroleum ether) to give *N*-{1-*tert*-butyl-3-[(1*S*,3*R*)-3-hydroxycyclopentyl]-1*H*-pyrazol-5-yl}-3-(methoxymethyl)-1-methyl-1*H*-pyrazole-5-carboxamide (13B, 8.0 g, 78%) as a yellow gum. MS: 376 [M+H]⁺.

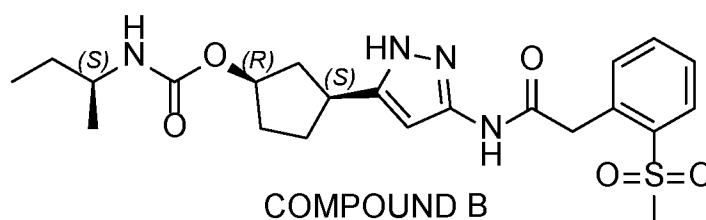
5 A solution of *N*-{1-*tert*-butyl-3-[(1*S*,3*R*)-3-hydroxycyclopentyl]-1*H*-pyrazol-5-yl}-3-(methoxymethyl)-1-methyl-1*H*-pyrazole-5-carboxamide (13B, 8.0 g, 21 mmol) in dichloromethane (80 mL) and THF (80 mL) was treated with DMAP (260 mg, 2.13 mmol), pyridine (5.06 g, 63.9 mmol), and 4-nitrophenyl chloroformate (8.59 g, 42.6 mmol). The resulting yellow suspension was stirred at room temperature for 18 hours. The reaction
10 mixture was concentrated to dryness and purified by silica gel chromatography (eluting with 0-45% ethyl acetate in petroleum ether) to give (1*R*,3*S*)-3-[1-*tert*-butyl-5-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-3-yl]cyclopentyl 4-nitrophenyl carbonate (13C, 10.6 g, 92%) as a light brown gum. MS: 541 [M+H]⁺.

A solution of (1*R*,3*S*)-3-[1-*tert*-butyl-5-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-
15 5-yl]carbonyl}amino)-1*H*-pyrazol-3-yl]cyclopentyl 4-nitrophenyl carbonate (13C, 10.6 g, 19.6 mmol) in formic acid (80 mL) was stirred at 70 °C for 18 hours. The solution was concentrated to dryness. The residue was dissolved in dichloromethane (150 mL) and the solution neutralized with sat. aq NaHCO₃. The organic layer was washed with water (30 mL) and sat. aq NaCl (30 mL), dried over sodium carbonate, filtered, and
20 concentrated to give crude (1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl 4-nitrophenyl carbonate (13D, 8.5 g, 90%, 86% pure by LCMS) as a light yellow glass. MS: 485 [M+H]⁺.

A room temperature (27 °C) solution of crude (1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-
1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl 4-nitrophenyl
25 carbonate (13D, 1.7 g, 3.5 mmol) and 2-propylamine (1.04 g, 17.5 mmol) in THF (30 mL) was stirred for 6 hours. The solution was concentrated to dryness, and the residue was combined with the residue from a second batch which had been derived from 1.7 g, 3.5 mmol 13D (total 6.27 mmol 13D consumed for the combined two batches) to give 3.2 g crude product. This product was purified by preparative HPLC on a Phenomenex Gemini
30 C18 250*50mm*10 μm column, eluting with 15-45% water (0.05% ammonium hydroxide v/v) in acetonitrile. After lyophilization, (1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate (COMPOUND A, 2.06 g, 78%) was obtained as a white crystalline solid monohydrate. MS: 405 [M+H]⁺. ¹H NMR (400MHz, DMSO-*d*₆) δ = 12.23 (br s, 1H), 10.73 (br s, 1H),
35 7.11 (s, 1H), 6.96 (br d, J=7.0 Hz, 1H), 6.41 (br s, 1H), 5.00 (br s, 1H), 4.33 (s, 2H), 4.04

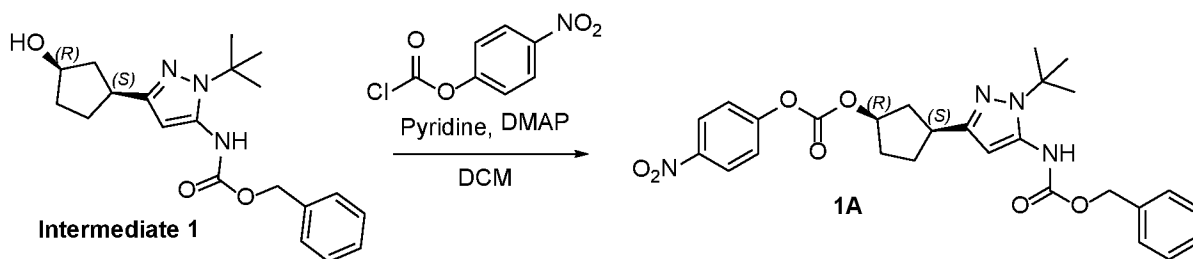
(s, 3H), 3.57 (qd, J=6.6, 13.4 Hz, 1H), 3.26 (s, 3H), 3.17-2.96 (m, 1H), 2.48-2.39 (m, 1H), 2.03 (br d, J=6.8 Hz, 1H), 1.95-1.83 (m, 1H), 1.73 (br d, J=8.5 Hz, 2H), 1.61 (br s, 1H), 1.03 (br d, J=6.3 Hz, 6H). Optical rotation $[\alpha]_D +4.8$ (c 1.0, MeOH). Chiral purity: >99% ee by chiral analytical SFC. Anal. Calcd for $C_{19}H_{28}N_6O_4 \cdot H_2O$: C, 54.02; H, 7.16; N, 19.89. Found: C, 53.94; H, 7.22; N, 19.81.

Example 5: Preparation of (1*R*,3*S*)-3-[3-({[2-(methylsulfonyl)phenyl]acetyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate (COMPOUND B)



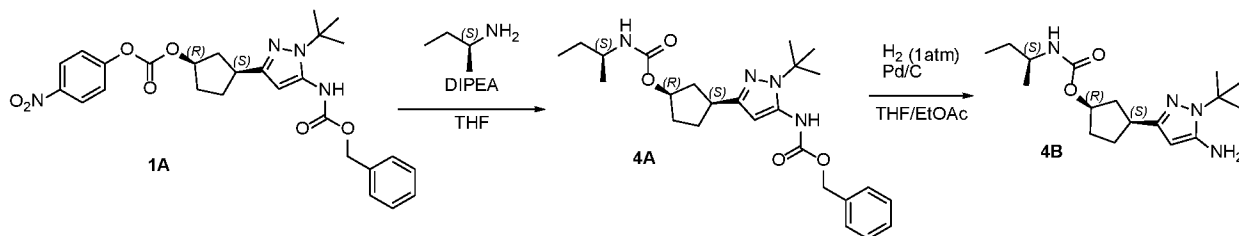
COMPOUND B was prepared as described below and characterized as in Example 370 U.S. 2020/0247784.

1. Preparation of Intermediate 1A: (1*R*,3*S*)-3-(5-({[(benzyloxy)carbonyl]amino}-1-*tert*-butyl-1*H*-pyrazol-3-yl)cyclopentyl 4-nitrophenyl carbonate



A room-temperature solution of benzyl {1-*tert*-butyl-3-[(1*S*,3*R*)-3-hydroxycyclopentyl]-1*H*-pyrazol-5-yl}carbamate (Intermediate 1, 5.00 g, 14.0 mmol) and 4-nitrophenyl chloroformate (4.23 g, 21.0 mmol) in anhydrous dichloromethane (50 mL) was treated with pyridine (3.40 mL, 42.0 mmol) and 4-(dimethylamino)pyridine (170 mg, 1.4 mmol). After stirring at room temperature overnight, the solution was concentrated and purified by silica gel chromatography (eluting with 0-100% ethyl acetate in n-heptane) to give (1*R*,3*S*)-3-(5-({[(benzyloxy)carbonyl]amino}-1-*tert*-butyl-1*H*-pyrazol-3-yl)cyclopentyl 4-nitrophenyl carbonate (1A, 7.30 g, 100%) as a solid foam. 1H NMR (400 MHz, CHLOROFORM-*d*) δ = 8.24-8.14 (m, 2H), 7.36-7.22 (m, 7H), 6.21 (br. s., 1H), 6.06 (br. s., 1H), 5.25 – 5.15 (m, 1H), 5.12 (s, 2H), 3.15-2.97 (m, 1H), 2.58-2.47 (m, 1H), 2.09-1.78 (m, 5H), 1.51 (s, 9H). MS: 523 $[M+H]^+$.

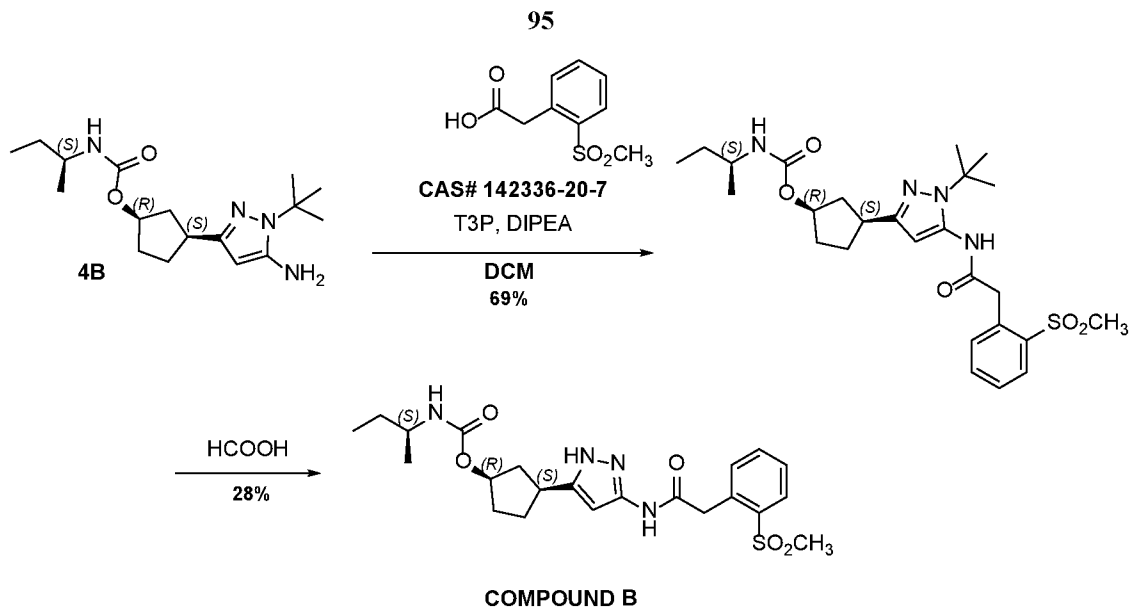
2. Preparation of Intermediate 4B: (1*R*,3*S*)-3-(5-amino-1-*tert*-butyl-1*H*-pyrazol-3-yl)cyclopentyl (2*S*)-butan-2-ylcarbamate



A solution of (1*R*,3*S*)-3-(5-[[*(benzyloxy)*carbonyl]amino]-1-*tert*-butyl-1*H*-pyrazol-3-yl)cyclopentyl 4-nitrophenyl carbonate (1A, 22.0 g, 42.1 mmol), (*S*)-(+)-*sec*-butylamine (4.00 g, 54.7 mmol), and diisopropylethyl amine (36.7 mL, 211 mmol) in THF (300 mL) was stirred at 10 °C for 16 hours. The mixture was concentrated to dryness, and the residue diluted with ethyl acetate (500 mL). The solution was washed with 1M aq NaOH (4 x 200 mL) and sat. aq NaCl (100 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated to give crude benzyl {3-[(1*S*,3*R*)-3-[(2*S*)-butan-2-ylcarbamoyl]oxy}cyclopentyl]-1-*tert*-butyl-1*H*-pyrazol-5-yl}carbamate (4A, 18 g, 94%, ~80% pure by LCMS). MS: 479 [M+Na]⁺.

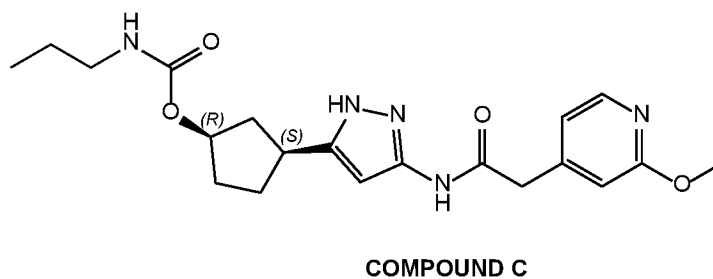
A room temperature (10°C) solution of the crude benzyl {3-[(1*S*,3*R*)-3-[(2*S*)-butan-2-ylcarbamoyl]oxy}cyclopentyl]-1-*tert*-butyl-1*H*-pyrazol-5-yl}carbamate (4A, 18 g, 39 mmol) in ethyl acetate (200 mL) and THF (100 mL) was degassed and treated with Pd/C catalyst (wet, 5 g). The suspension was stirred under a hydrogen balloon for 16 hours. The mixture was filtered to remove the catalyst, the filtrate was concentrated to dryness. For purification, this batch was combined with a second batch of crude derived by the same method from 20 g 4A (total for both batches: 38 g, 83 mmol) and purified by preparative HPLC on a Phenomenex Gemini C18 250*50mm*10 μm column, eluting with 30-50% water (0.05% ammonium hydroxide v/v) in acetonitrile. After lyophilization, (1*R*,3*S*)-3-(5-amino-1-*tert*-butyl-1*H*-pyrazol-3-yl)cyclopentyl (2*S*)-butan-2-ylcarbamate (4B, 20.1 g, 75% for the combined batches). MS: 323 [M+H]⁺. ¹H NMR (400MHz, DMSO-*d*₆) δ = 6.86 (br d, J=8.3 Hz, 1H), 5.22 (s, 1H), 4.94 (br s, 1H), 4.82-4.49 (m, 2H), 3.46-3.36 (m, 1H), 2.90-2.71 (m, 1H), 2.38-2.24 (m, 1H), 1.91-1.75 (m, 2H), 1.74-1.53 (m, 3H), 1.52-1.46 (m, 9H), 1.43-1.27 (m, 2H), 1.01 (d, J=6.5 Hz, 3H), 0.81 (t, J=7.4 Hz, 3H). Optical rotation [α]_D +4.0 (c 1.3, MeOH). Chiral purity: 98% de by chiral analytical SFC.

3. Preparation of COMPOUND B: (1*R*,3*S*)-3-[3-[[2-(methylsulfonyl)phenyl]acetyl]-amino]-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate



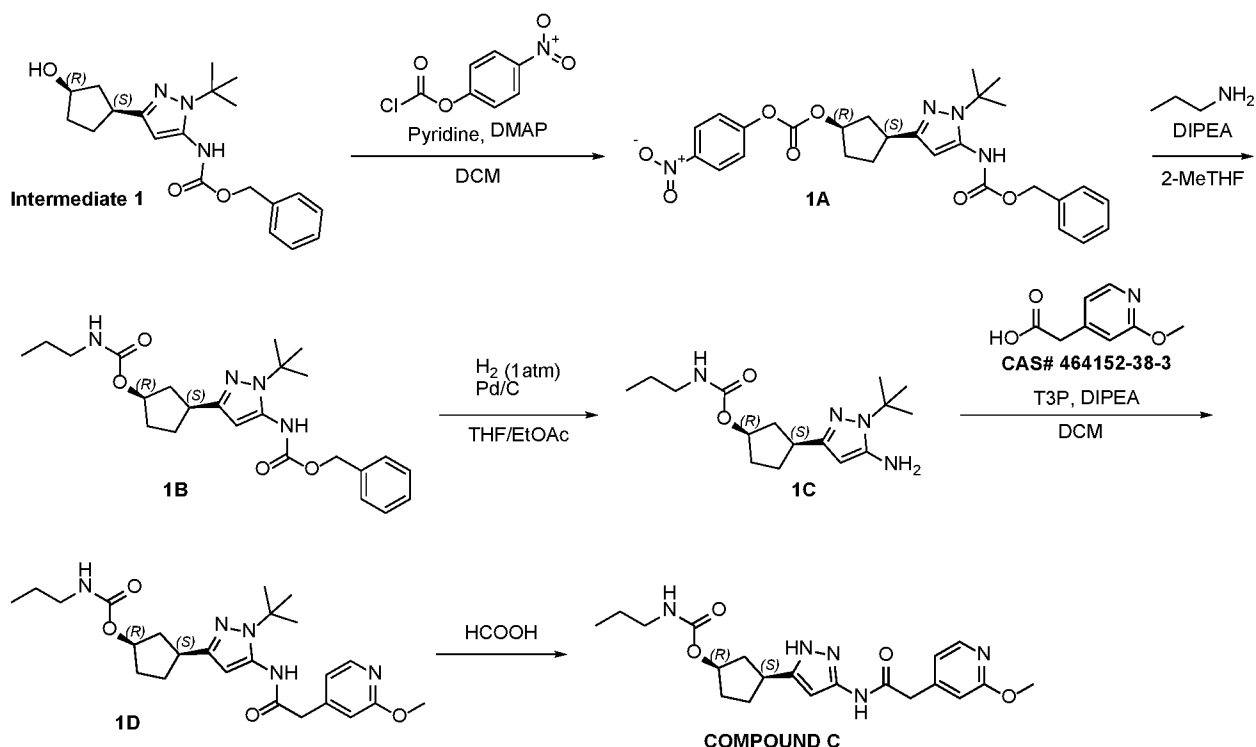
Intermediate 4B was converted to the 2-(methylsulfonyl)phenylacetamide intermediate under the conditions described in Example 6 (below) for conversion of Intermediate 1C to Intermediate 1D. The phenylacetamide intermediate was converted to COMPOUND B under the conditions described in Example 3 for conversion of Intermediate 1D to COMPOUND C.

Example 6: Preparation of (1R,3S)-3-(3-[(2-methoxypyridin-4-yl)acetyl]amino)-1H-pyrazol-5-yl)cyclopentyl propylcarbamate (COMPOUND C)



COMPOUND C was prepared as described in Example 1 of U.S. 2020/0247784.

1. Preparation of Compound C



A room-temperature solution of benzyl {1-*tert*-butyl-3-[(1*S*,3*R*)-3-hydroxycyclopentyl]-1*H*-pyrazol-5-yl}carbamate (Intermediate 1, 5.00 g, 14.0 mmol) and 4-nitrophenyl chloroformate (4.23 g, 21.0 mmol) in anhydrous dichloromethane (50 mL) was treated with pyridine (3.40 mL, 42.0 mmol) and 4-(dimethylamino)pyridine (170 mg, 1.4 mmol). After stirring at room temperature overnight, the solution was concentrated and purified by silica gel chromatography (eluting with 0-100% ethyl acetate in *n*-heptane) to give (1*R*,3*S*)-3-(5-[[[(benzyloxy)carbonyl]amino]-1-*tert*-butyl-1*H*-pyrazol-3-yl]cyclopentyl 4-nitrophenyl carbonate (1A, 7.30 g, 100%) as a solid foam. ¹H NMR (400 MHz, CHLOROFORM-*d*) δ = 8.24-8.14 (m, 2H), 7.36-7.22 (m, 7H), 6.21 (br. s., 1H), 6.06 (br. s., 1H), 5.25 – 5.15 (m, 1H), 5.12 (s, 2H), 3.15-2.97 (m, 1H), 2.58-2.47 (m, 1H), 2.09-1.78 (m, 5H), 1.51 (s, 9H). MS: 523 [M+H]⁺.

A solution of (1*R*,3*S*)-3-(5-[[[(benzyloxy)carbonyl]amino]-1-*tert*-butyl-1*H*-pyrazol-3-yl]cyclopentyl 4-nitrophenyl carbonate (1A, 36 g, 69 mmol) in 2-methyltetrahydrofuran (300 mL) was cooled to 10 °C. Diisopropylethyl amine (26.7 g, 36 mL, 207 mmol) and propan-1-amine (6.11 g, 8.52 mL, 103 mmol) were added, and the solution stirred at 10 °C for 16 hours. After concentrating to dryness, the residue was diluted with ethyl acetate (600 mL), washed with 1M NaOH (4 x 200 mL), and then with sat. aq NaCl (100 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated to give crude benzyl (1-*tert*-butyl-3-[(1*S*,3*R*)-3-[(propylcarbamoyl)oxy]cyclopentyl]-1*H*-pyrazol-5-yl)carbamate (1B, 30 g, 98%), which was used without further purification.

A room-temperature (20-25 °C) suspension of Pd/C (50% H₂O, 8 g) and crude benzyl (1-*tert*-butyl-3-((1*S*,3*R*)-3-[(propylcarbamoyl)oxy]cyclopentyl)-1*H*-pyrazol-5-yl)carbamate (1B, 30 g, 68 mmol) in ethyl acetate (300 mL) and THF (150 mL) was degassed and purged with hydrogen (3 cycles), then stirred at room temperature under a hydrogen balloon for 16 hours. The suspension was filtered, the filtrate concentrated under vacuum, and the residue crystallized from ethyl acetate (50 mL) and petroleum ether (300 mL), affording (1*R*,3*S*)-3-(5-amino-1-*tert*-butyl-1*H*-pyrazol-3-yl)cyclopentyl propylcarbamate (1C, 17.65 g, 84%) as a white solid. ¹H NMR (400MHz, DMSO-*d*₆) δ = 7.00 (br t, J=5.6 Hz, 1H), 5.23 (s, 1H), 4.95 (br s, 1H), 4.82-4.58 (m, 2H), 2.91 (q, J=6.6 Hz, 2H), 2.85-2.73 (m, 1H), 2.37-2.21 (m, 1H), 1.92-1.76 (m, 2H), 1.72-1.52 (m, 3H), 1.48 (s, 9H), 1.44-1.32 (m, 2H), 0.82 (t, J=7.4 Hz, 3H). MS: 309 [M+H]⁺. Optical rotation [α]_D -4.04 (c 0.89, MeOH). Chiral purity: 98% ee by chiral analytical SFC.

A cooled (10 °C) mixture of (1*R*,3*S*)-3-(5-amino-1-*tert*-butyl-1*H*-pyrazol-3-yl)cyclopentyl propylcarbamate (1C, 8.65 g, 28.05 mmol), (2-methoxypyridin-4-yl)acetic acid (CAS# 464152-38-3, 5.86 g, 33.7 mmol) diisopropylethyl amine (14.7 mL, 84.1 mmol) and propylphosphonic anhydride (T3P®, 50 wt% solution in EtOAc, 53.5 g, 84.1 mmol) in dichloromethane (250 mL) was stirred for 16 hours. The reaction was quenched with sat. aq Na₂CO₃ (20 mL) and extracted with dichloromethane (100 mL). The organic layer was washed with more sat. aq Na₂CO₃ (2 x 200 mL) and sat. aq NaCl (100 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. For purification, this batch was combined with two other similarly-prepared batches derived from 1.0 g and 8.0 g 1C (total SM for the three batches = 17.65 g, 57.23 mmol 1C). Silica gel chromatography (eluting with 0-60% EtOAc/petroleum ether) gave (1*R*,3*S*)-3-(1-*tert*-butyl-5-[(2-methoxypyridin-4-yl)acetyl]amino)-1*H*-pyrazol-3-yl)cyclopentyl propylcarbamate (1D, 25 g, 95% yield for the combined batches). MS: 458 [M+H]⁺.

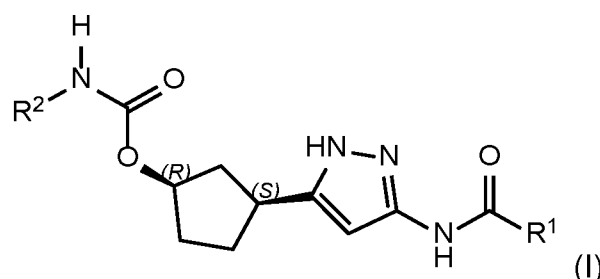
A solution of (1*R*,3*S*)-3-(1-*tert*-butyl-5-[(2-methoxypyridin-4-yl)acetyl]amino)-1*H*-pyrazol-3-yl)cyclopentyl propylcarbamate (1D, 20.5 g, 44.8 mmol) in formic acid (50 mL) was stirred at 75 °C for 20 hours. For purification, this batch was combined with a smaller batch (derived from 4.50 g, 9.84 mmol 1D, for a total of 25.0 g, 54.6 mmol), concentrated to dryness, and purified by preparative HPLC [Phenomenex Gemini C18 250 x 50mm x 10 μm column; eluting with a gradient of water (0.05% ammonium hydroxide v/v) in ACN over 15 minutes; flowing at 110 mL/min]. Pure (1*R*,3*S*)-3-(3-[(2-methoxypyridin-4-yl)acetyl]amino)-1*H*-pyrazol-5-yl)cyclopentyl propylcarbamate (COMPOUND C), 16.61 g, 76% yield for the combined batches) as a pale yellow solid. ¹H NMR (400 MHz, CHLOROFORM-*d*) δ = 11.62-9.81 (m, 1H), 9.06 (br s, 1H), 8.06 (d, J=5.3 Hz, 1H), 6.79

(d, J=5.3 Hz, 1H), 6.66 (s, 1H), 6.50 (s, 1H), 5.24-4.94 (m, 2H), 3.88 (s, 3H), 3.58 (s, 2H), 3.19-2.83 (m, 3H), 2.54-2.28 (m, 1H), 2.04 (br s, 1H), 1.97-1.70 (m, 4H), 1.54-1.34 (m, 2H), 0.85 (br t, J=7.0 Hz, 3H). MS: 402 [M+H]⁺. Optical rotation [α]_D +17.1 (c 1.06, MeOH). Chiral purity: 96% ee by chiral analytical SFC.

CLAIMS

1. A method for treating cancer comprising administering to a subject in need thereof, an amount of a cyclin dependent kinase 2 (CDK2) inhibitor in combination with an amount of a cell division cycle 25A (CDC25A) inhibitor, wherein the amounts together are effective
5 in treating cancer.

2. The method of claim 1, wherein the CDK2 inhibitor is a compound of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

R¹ is -L-(5-6 membered heteroaryl) or -L-(phenyl), where said 5-6 membered
10 heteroaryl or phenyl is optionally substituted by one to three R³;

R² is C₁-C₆ alkyl or C₃-C₇ cycloalkyl, where said C₃-C₇ cycloalkyl is optionally substituted by C₁-C₄ alkyl;

L is a bond or a methylene; and

each R³ is independently C₁-C₄ alkyl, C₁-C₄ alkoxy or SO₂-C₁-C₄ alkyl, where each
15 C₁-C₄ alkyl is optionally substituted by F, OH or C₁-C₄ alkoxy.

3. The method of claim 1 or 2, wherein the CDK2 inhibitor is selected from the group consisting of:

(1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate;

(1*R*,3*S*)-3-[3-({[2-(methylsulfonyl)phenyl]acetyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate; and
20

(1*R*,3*S*)-3-(3-[(2-methoxypyridin-4-yl)acetyl]amino)-1*H*-pyrazol-5-yl]cyclopentyl propylcarbamate, or a pharmaceutically acceptable salt thereof.

4. The method of any one of claims 1 to 3, wherein the CDK2 inhibitor is (1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate.
25

5. The method of claim 1, wherein the CDK2 inhibitor is 6-(difluoromethyl)-8-((1R,2R)-2-hydroxy-2-methylcyclopentyl)-2-(1-(methylsulfonyl)piperidin-4-ylamino)pyrido[2,3-d]pyrimidin-7(8H)-one, or a pharmaceutically acceptable salt thereof.

6. The method of any one of claims 1 to 5, wherein the CDC25A inhibitor comprises a small molecule inhibitor, a small molecule degrader, a polypeptide, a peptide, a glycoprotein, a peptidomimetic, an antigen binding construct, a nucleic acid, a genetic construct for targeted gene editing, an antibody-like protein scaffold, an aptamer, or a combination thereof.

7. The method of claim 6, wherein the nucleic acid is small interfering RNA (siRNA), short hairpin RNA (shRNA), antisense or inhibitory DNA or RNA, ribozyme, RNA or DNA aptamer, RNAi, or peptide nucleic acid (PNA), or a combination thereof.

8. The method of claim 6, wherein the genetic construct for targeted gene editing is CRISPR/Cas9 construct, guide RNA (gRNA), guide DNA (gDNA) or tracrRNA, or combination thereof.

9. The method of claim 6, wherein the polypeptide is an antibody or antibody fragment thereof.

10. The method of claim 6, wherein the small molecule inhibitor is 2-(2-mercaptoethanol)-3-methyl-1,4-naphthoquinone, 1-([1,1'-biphenyl]-4-yl)-3,4-bis((2-hydroxyethyl)thio-1*H*-pyrrole-2,5-dione (PM-20), 2-(2,5-difluorophenyl)-6-((3-(methyl(3-((2-methyl-4,7-dioxo-4,7-dihydrobenzo[d]thiazol-5-yl)amino)propyl)amino)propyl)amino)benzo[d]oxazole-4,7-dione (IRC 083864), or 2-methoxyestadiol, or a pharmaceutically acceptable salt thereof.

11. The method of any one of claims 1 to 10, wherein the subject is a human.

12. The method of any one of claims 1 to 11, wherein the cancer is selected from the group consisting of lung cancer, breast cancer, brain cancer, head and neck cancer, prostate cancer, ovarian cancer, bladder cancer, bone cancer, colorectal cancer, kidney cancer, liver cancer, stomach cancer, pancreatic cancer, esophageal cancer, cervical cancer, sarcoma, skin cancer, mesothelioma, malignant rhabdoid tumors, neuroblastoma, and diffuse intrinsic pontine glioma (DIPG).

13. The method of any one of claims 1 to 11, wherein the cancer is a cyclin E dominant cancer.

14. The method of claim 13, wherein the cyclin E dominant cancer is ovarian cancer, breast cancer, prostate cancer, small cell lung cancer, colorectal cancer or
5 pancreatic cancer.

15. The method of any one of claims 1 to 11, wherein the cancer is characterized by loss of RB.

16. The method of claim 15, wherein the cancer is breast cancer, small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNEC), or neuroendocrine prostate
10 cancer (NEPC).

17. The method of any one of claims 1 to 16, wherein the CDK2 inhibitor and the CDC25A inhibitor are administered simultaneously, sequentially or separately.

18. A combination comprising:

a. (i) (1*R*,3*S*)-3-[3-({[3-(methoxymethyl)-1-methyl-1*H*-pyrazol-5-yl]carbonyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl propan-2-ylcarbamate, and (ii) a
15 CDC25A inhibitor;

b. (i) (1*R*,3*S*)-3-[3-({[2-(methylsulfonyl)phenyl]acetyl}amino)-1*H*-pyrazol-5-yl]cyclopentyl (2*S*)-butan-2-ylcarbamate, and (ii) a CDC25A inhibitor;

c. (i) (1*R*,3*S*)-3-(3-[(2-methoxypyridin-4-yl)acetyl]amino)-1*H*-pyrazol-5-yl]cyclopentyl propylcarbamate, and (ii) a CDC25A inhibitor; or
20

d. (i) 6-(difluoromethyl)-8-((1*R*,2*R*)-2-hydroxy-2-methylcyclopentyl)-2-(1-(methylsulfonyl)piperidin-4-ylamino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one, or a pharmaceutically acceptable salt thereof; and (ii) a CDC25A inhibitor;
for use in the treatment of cancer.

19. The combination of claim 18, wherein the cancer is selected from the group consisting of lung cancer, breast cancer, brain cancer, head and neck cancer, prostate cancer, ovarian cancer, bladder cancer, bone cancer, colorectal cancer, kidney cancer, liver cancer, stomach cancer, pancreatic cancer, esophageal cancer, cervical cancer, sarcoma, skin cancer, mesothelioma, malignant rhabdoid tumors, neuroblastoma, and
30 diffuse intrinsic pontine glioma (DIPG).

20. The combination of claim 18, wherein the cancer is a cyclin E dominant cancer.

21. The combination of claim 20, wherein the cyclin E dominant cancer is ovarian cancer, breast cancer, prostate cancer, small cell lung cancer, colorectal cancer or pancreatic cancer.

5 22. The combination of claim 18, wherein the cancer is characterized by loss of RB.

23. The combination of claim 22, wherein the cancer is breast cancer, small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNEC), or neuroendocrine prostate cancer (NEPC).

FIG. 1

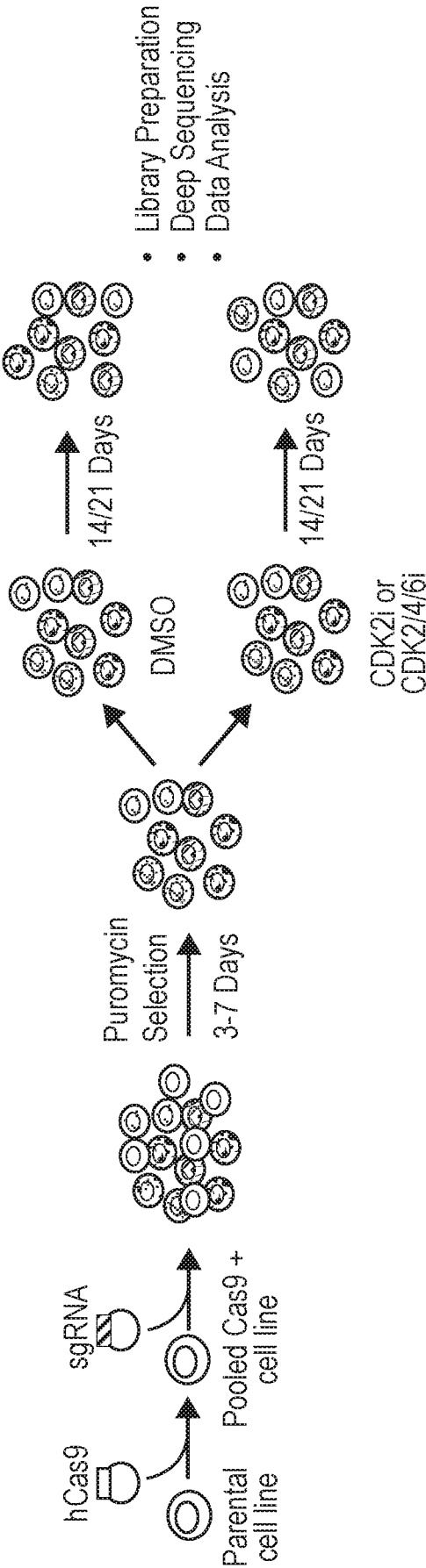
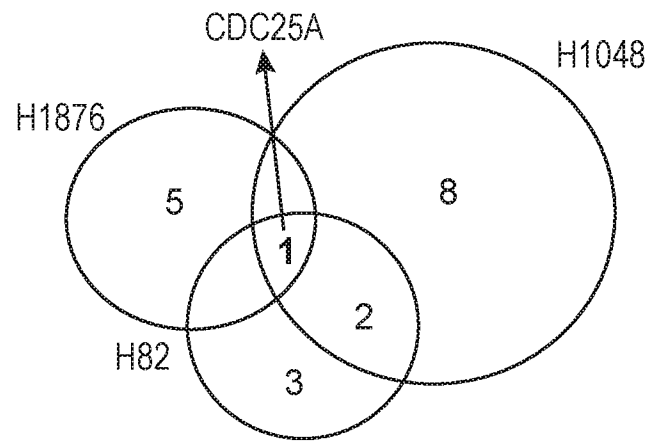


FIG. 2A

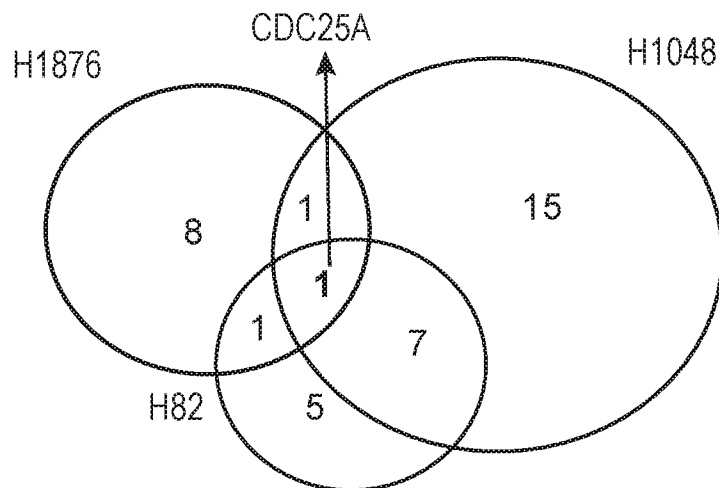
CDK2i



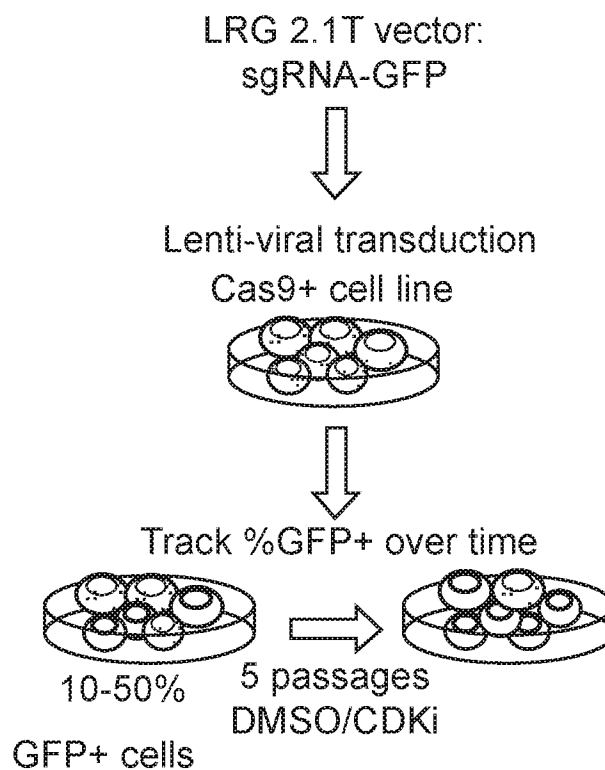
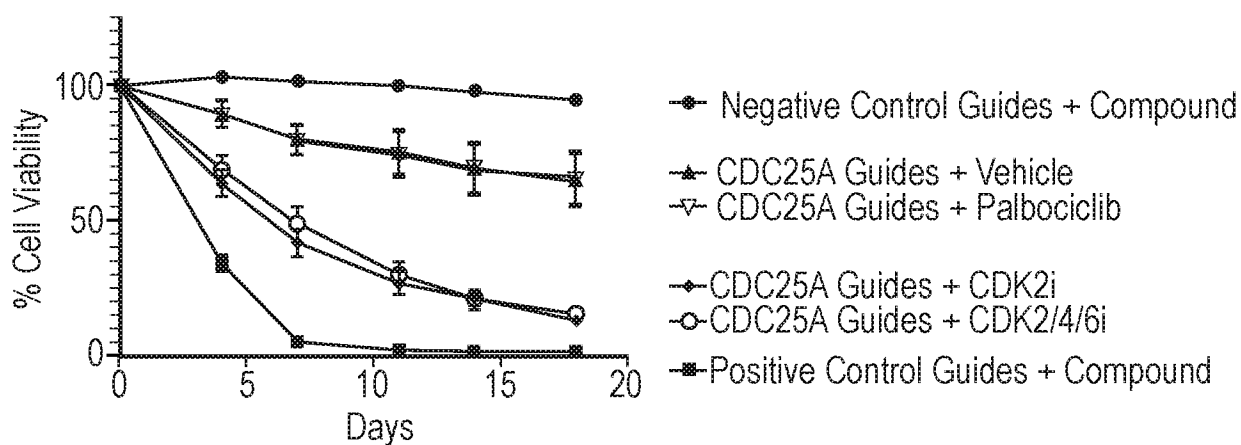
FDR<0.1
Beta score<0

FIG. 2B

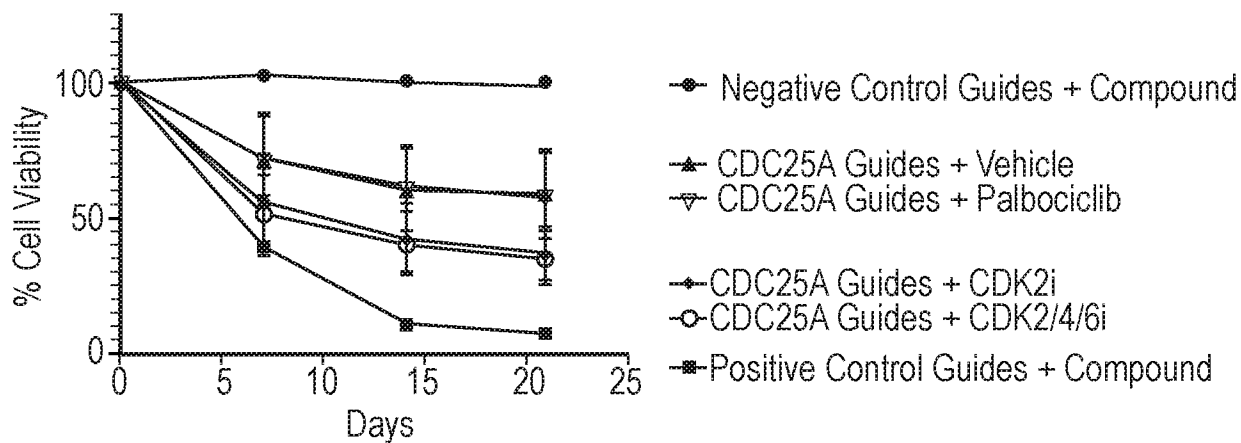
CDK2/4/6i



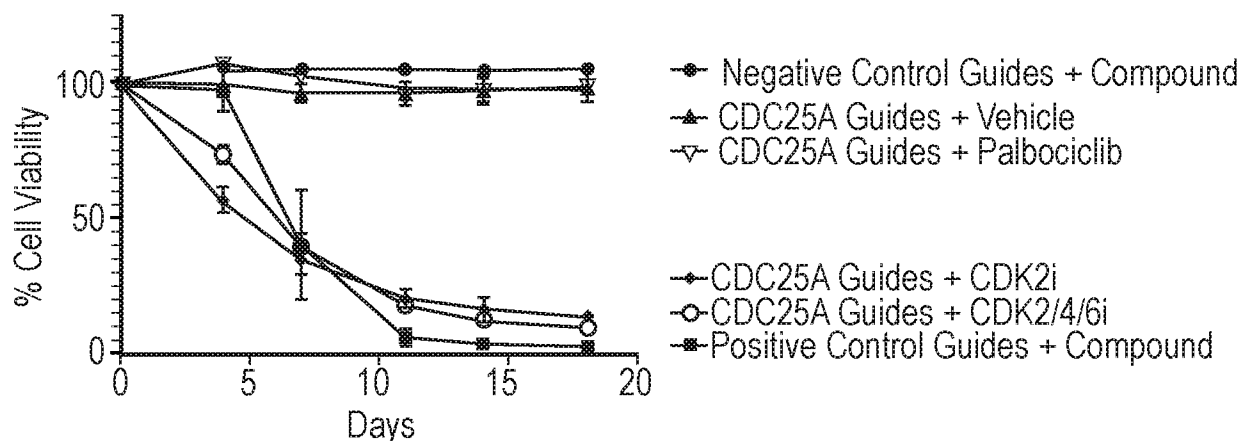
FDR<0.1
Beta score<0

FIG. 3A**GFP competition assay****FIG. 3B****CDC25A Drop Out Validation in H1048**

CDK2i: 150 nM
 CDK2/4/6i: 50 nM
 Palbociclib (CDK4/6i): 150 nM

FIG. 3C**CDC25A Drop Out Validation in H1876**

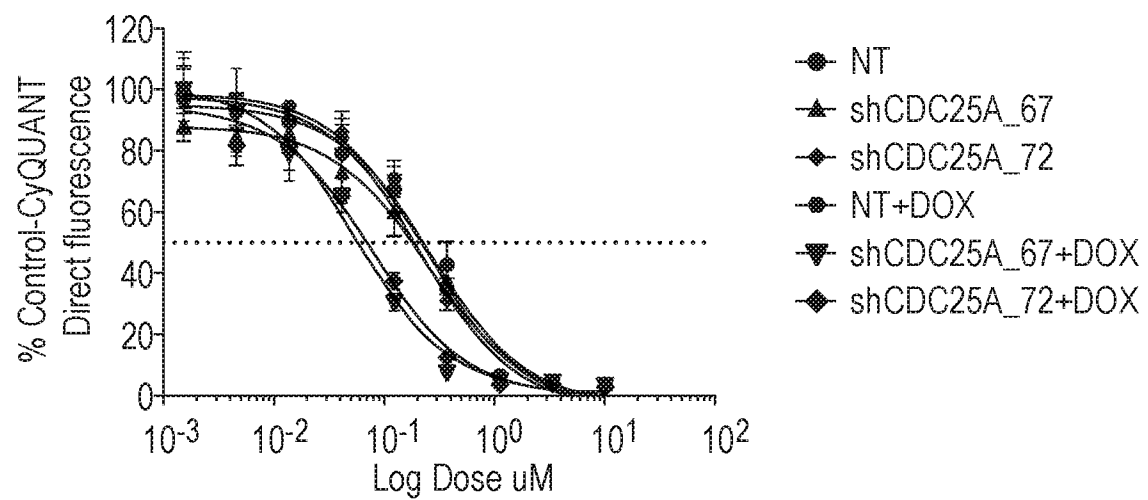
CDK2i: 30 nM
CDK2/4/6i: 10 nM
Palbociclib (CDK4/6i): 150 nM

FIG. 3D**CDC25A Drop Out Validation in H211**

CDK2i: 100 nM
CDK2/4/6i: 30 nM
Palbociclib (CDK4/6i): 30 nM

FIG. 4A

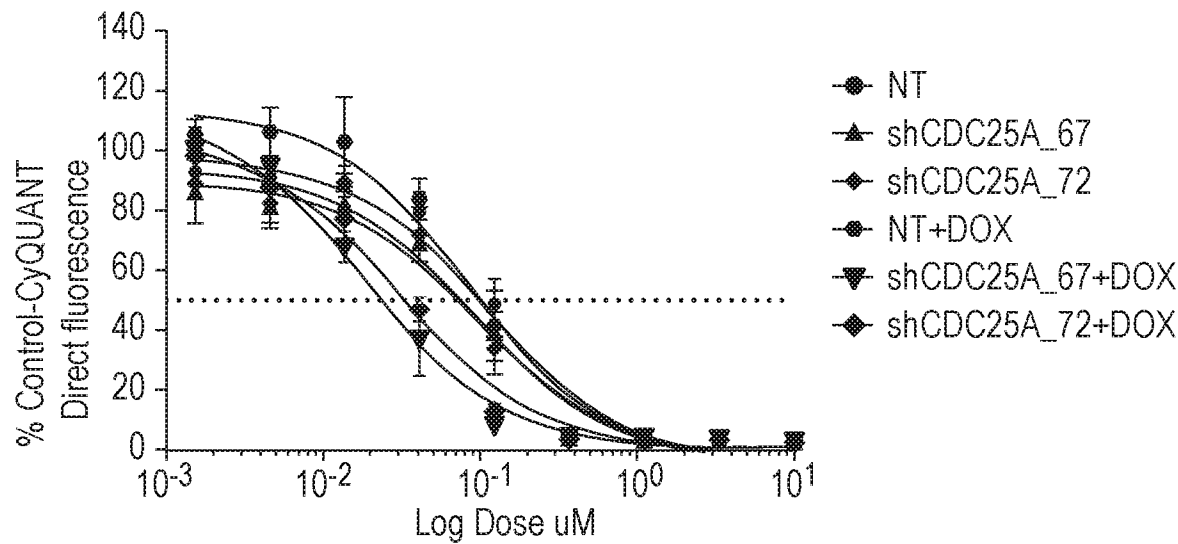
Anti-proliferation effects in H82 with CDK2i



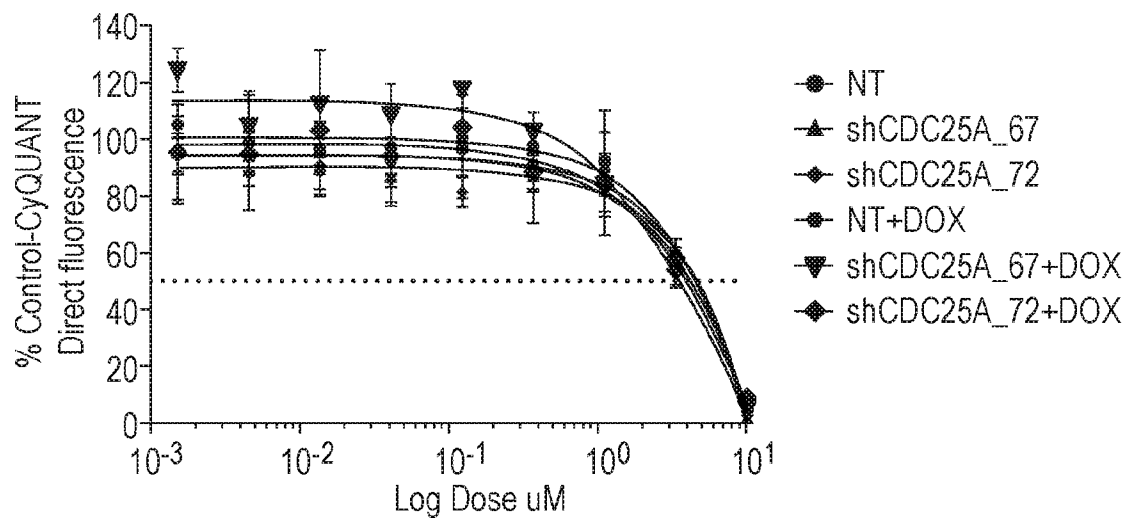
		IC50 (uM)	Compound A
-Dox	NT	0.2702	
	shCDC25A_67	0.2458	
	shCDC25A_72	0.2167	
+Dox	NT+DOX	0.2387	4-fold
	shCDC25A_67+DOX	0.05673	
	shCDC25A_72+DOX	0.07816	3-fold

FIG. 4B

Anti-proliferation effects in H82 with CDK2/4/6i



		IC50 (uM)	PF-06873600		
-Dox		NT	0.1138	4-fold	
		shCDC25A_67	0.09631		
		shCDC25A_72	0.08817		
+Dox		NT+DOX	0.08384	3-fold	
		shCDC25A_67+DOX	0.01943		
		shCDC25A_72+DOX	0.03111		

FIG. 4C**Anti-proliferation effects in H82 with CDK4/6i**

		IC50 (uM)	Palbociclib
-Dox	NT	>3	
	shCDC25A_67	>3	
	shCDC25A_72	>3	
+Dox	NT+DOX	>3	
	shCDC25A_67+DOX	>3	
	shCDC25A_72+DOX	>3	

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2021/056604

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/415 A61K31/4155 A61K31/519 A61K45/06 A61P35/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

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

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

EP0-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	US 2017/112809 A1 (ORWAR OWE [SE] ET AL) 27 April 2017 (2017-04-27) paragraph [0067] paragraph [0073] - paragraph [0075] paragraph [0082] ----- -/--	1,6,11, 12,17 2-5, 7-10, 13-16, 18-23



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

28 October 2021

Date of mailing of the international search report

12/11/2021

Name and mailing address of the ISA/

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

Authorized officer

Büttner, Ulf

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2021/056604

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LAVECCHIA ANTONIO ET AL: "Inhibitors of Cdc25 phosphatases as anticancer agents: a patent review", EXPERT OPINION ON THERAPEUTIC PATENTS, vol. 20, no. 3, 24 March 2010 (2010-03-24) , pages 405-425, XP055848033, GB ISSN: 1354-3776, DOI: 10.1517/13543771003623232 Retrieved from the Internet: URL:http://dx.doi.org/10.1517/13543771003623232>	1-9, 11-23
A	page 421, column 1, paragraph 2	10
A	TATYANA V. MASYUK ET AL: "Inhibition of Cdc25A Suppresses Hepato-renal Cystogenesis in Rodent Models of Polycystic Kidney and Liver Disease", GASTROENTEROLOGY, vol. 142, no. 3, 1 March 2012 (2012-03-01) , pages 622-633.e4, XP055114269, ISSN: 0016-5085, DOI: 10.1053/j.gastro.2011.11.036 page 622, column 2	1-23
A	----- W0 2018/033815 A1 (PFIZER [US]) 22 February 2018 (2018-02-22) claim 1	1-23
A	----- LIU QI ET AL: "To control or to be controlled? Dual roles of CDK2 in DNA damage and DNA damage response", DNA REPAIR, ELSEVIER, AMSTERDAM, NL, vol. 85, 16 September 2019 (2019-09-16), XP086044093, ISSN: 1568-7864, DOI: 10.1016/J.DNAREP.2019.102702 [retrieved on 2019-09-16] page 3, column 1	1-23
A,P	----- US 2020/247784 A1 (BEHENNA DOUGLAS CARL [US] ET AL) 6 August 2020 (2020-08-06) paragraph [0009] claim 1 -----	1-23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2021/056604

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

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2021/056604

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2017112809 A1	27-04-2017	DK 3148532 T3 EP 3148532 A1 US 2017112809 A1 US 2020138782 A1 WO 2015181737 A1	26-04-2021 05-04-2017 27-04-2017 07-05-2020 03-12-2015
WO 2018033815 A1	22-02-2018	AU 2017311645 A1 BR 112019002610 A2 CA 2975033 A1 CL 2019000368 A1 CN 109803968 A CO 2019001240 A2 CR 20190062 A CU 20190010 A7 DK 3497103 T3 DO P2019000030 A EC SP19011216 A EP 3497103 A1 GE P20217234 B HR P20210871 T1 IL 264687 A JP 6563623 B1 JP 2019527722 A KR 20190038915 A LT 3497103 T MA 45920 A MD 3497103 T2 NI 201900013 A PE 20190475 A1 PH 12019500329 A1 PT 3497103 T RU 2726115 C1 SG 11201900799X A SI 3497103 T1 SV 2019005836 A TN 2019000039 A1 TW 201819383 A US 2018044344 A1 US 2019135817 A1 US 2020392142 A1 UY 37352 A WO 2018033815 A1	28-02-2019 02-07-2019 15-02-2018 10-05-2019 24-05-2019 19-02-2019 22-05-2019 04-10-2019 14-06-2021 30-06-2019 29-03-2019 19-06-2019 25-03-2021 09-07-2021 29-04-2021 21-08-2019 03-10-2019 09-04-2019 26-07-2021 19-06-2019 31-08-2021 08-04-2019 04-04-2019 28-10-2019 17-06-2021 09-07-2020 27-02-2019 30-07-2021 19-03-2019 15-07-2020 01-06-2018 15-02-2018 09-05-2019 17-12-2020 23-03-2018 22-02-2018
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2021/056604

Patent document cited in search report	Publication date	Patent family member(s)	Publication date