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(54) Title: CHK1(SRA737)WEE1 INHIBITOR COMBINATION METHODS OF INHIBITING TUMOR GROWTH

(57) Abstract: Herein disclosed are combinations of Checkpoint kinase 1 (Chkl) inhibitors and Weel inhibitors useful for inhibiting the growth of tumors such as those in patients with cancer. In particular, the combination demonstrates remarkable synergistic effects on cancer cells that are representative models for tumors. Also provided for are methods for treating disorders or disease mediated or affected by Chkl, CDKl/2 and/or CDC25 activity.

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CHK1(SRA737)/WEE1 INHIBITOR COMBINATION METHODS OF INHIBITING TUMOR GROWTH

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/483,888 filed April 10, 2017; the contents of which are incorporated herein in its entirety by reference thereto.

BACKGROUND OF THE INVENTION

Field of the invention

[0002] The invention relates to methods, compositions and kits useful for inhibiting tumor growth. In particular, disclosed herein are combinations of Checkpoint kinase 1 (Chk1) inhibitors and Wee1 inhibitors useful for pharmaceutical compositions, kits and methods of inhibiting tumor growth, *e.g.*, tumor growth relating to cancer.

Description of the Related Art

[0003] Cells activate a signal transduction pathway when DNA is damaged. Signals activate the cell-cycle machinery to induce DNA repair and/or cell death to mitigate propagation. Checkpoint kinase 1 (Chkl) is an important bridge in cells when sensing DNA damage. See Cancer Biology & Therapy (2004) 3:3, 305-313, incorporated herein by reference. Chk1 plays a role in regulating numerous and wide-ranging cellular functions including: immune and inflammation responses, spindle formation, DNA damage signal transduction and generally, cellular apoptosis. Chk1 inhibitors abrogate DNA damage-induced cell cycle arrest in S and/or G 2/M phases. Currently, there are no Chk1 inhibitors that are approved therapies for treatment of inhibition of tumor growth.

SUMMARY OF THE INVENTION

[0004] In certain aspects, described herein are methods of inhibiting the growth of a tumor in a subject in need thereof, comprising administering to the subject a first effective amount of a

Chk1 inhibitor and a second effective amount of a Wee1 inhibitor. In certain aspects, described herein are methods of inhibiting the growth of a tumor in a subject in need thereof, comprising administering to the subject a first effective amount of a SRA737 and a second effective amount of a Wee1 inhibitor. In certain embodiments, the SRA737 and the Wee1 inhibitor are administered separately. In certain embodiments, the Wee1 inhibitor is administered at least twenty-four (24) hours after the administration of SRA737. In certain embodiments, the SRA737 and the Wee1 inhibitor are administered; and subsequently both SRA737 and the Wee1 inhibitor are administered continuously for at least twenty-four (24) hours. In certain embodiments, the SRA737 and the Wee1 inhibitor are administered; and subsequently either one of, or both of, SRA737 and/or the Wee1 inhibitor is separately administered intermittently for at least twenty-four (24) hours. In certain embodiments, the Wee1 inhibitor is selected from the group consisting of: MK 1775, PD166285, PF00120130, 4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3-(2H,6H)-dione (PHCD), ADC-730, ADC-999, and PD0407824. In certain embodiments, the Wee1 inhibitor is MK 1775. In certain embodiments, the tumor is in a subject suffering from a condition or disorder selected from the group consisting of: bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, mesothelioma, melanoma, myeloma, ovarian cancer, prostate cancer, pancreatic cancer, renal cell cancer, small cell lung cancer, and squamous cell carcinoma of the head and neck. In certain embodiments, the subject has a mutation in at least one gene selected from the group consisting of: a tumor suppressor gene, a DNA damage repair gene, a replication stress gene, or an oncogenic driver gene. In certain embodiments, the route of administration is selected from the group consisting of: intravenous, subcutaneous, cutaneous, oral, intramuscular, and intraperitoneal. In certain embodiments, the first effective amount is 0.001 mg/kg to 15 mg/kg and the second effective amount is 0.001 mg/kg to 15 mg/kg. In certain embodiments, the first effective amount is 0.1 mg/kg to 1.5 mg/kg and the second effective amount is 0.1 mg/kg to 1.5 mg/kg. In certain embodiments, the first effective amount is 10 mg to 1000 mg. In certain embodiments, tumor growth is reduced in the subject. In certain embodiments, tumor growth is reduced by at least 1% after administration. In certain embodiments, administration results in tumor growth of no more than 5% of the original tumor volume after administration. In certain embodiments, the subject is human.

[0005] In certain aspects, described here are methods of inhibiting a Chk1 activity in a cell, the method comprising contacting the cell with a first effective amount of SRA737 and a second effective amount of a Wee1 inhibitor. In certain aspects, described here are methods of activating CDC25 activity in a cell, the method comprising contacting the cell with a first effective amount of SRA737 and a second effective amount of a Wee1 inhibitor. In certain aspects, described here are methods of activating CDK1/2 activity in a cell, the method comprising contacting the cell with a first effective amount of SRA737 and a second effective amount of a Wee1 inhibitor. In an embodiment, the cell is a tumor cell. In an embodiment, the method is performed *in vitro*. In certain embodiments, the SRA737 and the Wee1 inhibitor are administered simultaneously. In certain embodiments, the SRA737 and the Wee1 inhibitor are administered sequentially. In certain embodiments, the SRA737 and the Wee1 inhibitor are administered; and subsequently both SRA737 and the Wee1 inhibitor are administered continuously for at least twenty-four (24) hours. In certain embodiments, the SRA737 and the Wee1 inhibitor are administered; and subsequently either one of, or both of: SRA737 or the Wee1 inhibitor is separately administered intermittently for at least twenty-four (24) hours.

[0006] In certain aspects, described herein is a combination comprising SRA737 and a Wee1 inhibitor. In certain embodiments, the Wee1 inhibitor is selected from the group consisting of: MK 1775, PD166285, PF00120130, 4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3-(2H,6H)-dione (PHCD), ADC-730, ADC-999, and PD0407824. In certain aspects, described herein are pharmaceutical compositions comprising the combinations comprising SRA737 and a Wee1 inhibitor and at least one pharmaceutically acceptable carrier or excipient.

[0007] In an aspect, described herein is a SRA737 for use in inhibiting a tumor growth in a subject in need thereof by co-administration with a Wee1 inhibitor. In an aspect, described herein is a Wee1 inhibitor for use in inhibiting a tumor growth in a subject in need thereof by co-administration with SRA737. In an aspect, described herein is a product comprising SRA737 and a Wee1 inhibitor for simultaneous, separate or sequential use in the inhibition of a tumor growth in a subject in need thereof.

[0008] In certain aspects, described herein are methods of inhibiting a tumor growth in a subject in need thereof, comprising administering to the subject a first effective amount of a Chk1 inhibitor and a second effective amount of Wee1 inhibitor. In certain embodiments, the

Wee1 inhibitor is selected from the group consisting of: MK 1775, PD166285, PF00120130, 4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3-(2H,6H)-dione (PHCD), ADC-730, ADC-999, and PD0407824. In certain embodiments, the subject has a condition or disorder selected from the group consisting of: bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, mesothelioma, melanoma, myeloma, ovarian cancer, prostate cancer, pancreatic cancer, renal cell cancer, small cell lung cancer, and squamous cell carcinoma of the head and neck.

[0009] In an aspect, described is a Chk1 inhibitor for use in inhibiting a tumor growth in a subject in need thereof by co-administration with a Wee1. In an aspect, described herein is a Wee1 inhibitor for use in inhibiting a tumor growth in a subject in need thereof by co-administration with a Chk1 inhibitor. In an aspect, described herein is a product comprising a Chk1 inhibitor and a Wee1 inhibitor for simultaneous, separate or sequential use in the inhibition of a tumor growth in a subject in need thereof. In an aspect, described herein are methods comprising use of a Chk1 inhibitor in the manufacture of a medicament for treatment tumor growth in a subject in need thereof by co-administration of a Wee1 inhibitor. In an aspect, described herein are methods comprising use of a Wee1 inhibitor in the manufacture of a medicament for treatment tumor growth in a subject in need thereof by co-administration of a Chk1 inhibitor. In certain embodiments, the Chk1 inhibitor is selected from the group consisting of Prexasertib (LY2606368), PF-477736, AZD7762, Rabusertib (LY2603618), MK-8776 (SCH 900776), CHIR-124, SAR-020106 and CCT245737. In an embodiment, the Chk1 inhibitor is Prexasertib (LY2606368). In an embodiment, the Chk1 inhibitor is PF-477736. In an embodiment, the Chk1 inhibitor is AZD7762. In an embodiment, the Chk1 inhibitor is Rabusertib (LY2603618). In an embodiment, the Chk1 inhibitor is MK-8776 (SCH 900776). In an embodiment, the Chk1 inhibitor is CHIR-124. In an embodiment, the Chk1 inhibitor is SAR-020106. In an embodiment, the Chk1 inhibitor is CCT245737.

[0010] In certain aspects, described herein are kits comprising the combinations or the pharmaceutical compositions combinations comprising SRA737 and a Wee1 inhibitor and instructions for use.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0011] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

[0012] **Figure 1**: A schematic presentation of the co-treatment and sequential treatment dosing schedules for combination studies is shown. In the co-treatment schedule, both enhancee (*i.e.*, SRA737 also referred to as "ProNAi Compound 1" or "Sierra Compound 1") and enhancer (partner compound, *i.e.*, the Wee1 inhibitor MK 1775) were added at time zero. In the sequential treatment schedule, only the enhancer was added at time zero. The enhancee was added 24 hours after the addition of enhancer. All other assay conditions were identical in both dosing schedules.

[0013] **Figure 2:** Single agent dose response profiles to SRA737 "ProNAi Compound 1" across the cell line panel is shown.

[0014] **Figure 3:** A diagram of methods for quantification of Synergy Score and Loewe Volume using the Loewe Additivity model is shown. Synergy can be quantified in relation to the Loewe additivity shape model (Null Interaction Model) which is constructed from the single agent dose responses. The additivity model serves as a "null-hypothesis" and assumes no synergistic interaction between the enhancee (SRA737 "ProNAi compound 1") and the enhancer (Gemcitibine Hydrochloride). To quantify Loewe Volume, the empiric data surface is subtracted from the additivity shape model (Null Interaction Model). Loewe Volume is the summation of any residual excess activity across the combination dose matrix and can be used to measure both potential synergy (positive Loewe Volume) and antagonism (negative Loewe Volume). Synergy Score is a positively gated value, and cannot be used to gauge potential antagonism. Scores in this figure were calculated using the logistic curve fit.

[0015] **Figure 4:** A 6 x 6 optimized dose matrix format for combination treatment growth inhibition results is shown. The enhancer single agent dose curve is shown on the vertical axis. The enhancee single agent dose curve is shown along the horizontal axis. For each 6 x 6 dose matrix, the enhancee (SRA737 "ProNAi compound 1") and enhancer (Gemcitabine Hydrochloride) are collected in a single agent dose series of 5 points (plus the zero) and a total of 13 combination dose ratio points are collected.

[0016] **Figure 5**: A Synergy Score heat map for SRA737 (ProNAi Compound 1) in combination with DNA damage response inhibitors (the Wee1 inhibitor, MK 1775 and the DNA-PK inhibitor, NU7441) in both the co-treatment and the sequential treatments. A Synergy Score cut-off of 3.0 was made for the analysis of the co-treatment schedule with Synergy Scores from 3.0-6.0 highlighted in lighter shades of grey while scores above 6.0 are highlighted in dark grey. A Synergy Score cut-off of 3.0 was made for the analysis of the sequential treatment schedule with Synergy Scores from 3.0-4.5 highlighted in lighter shades of grey while scores above 4.5 are highlighted in dark grey.

[0017] **Figure 6:** The Growth Inhibition dose matrices are shown for cell lines with the two highest Synergy Scores in the co-treatment and co-treatment dosing schedules. Upper row: results are shown from the co-treatment schedule for the bladder cancer cell line J82 (left) and the pancreatic cancer cell line, MIA PaCa-2 (right). Lower row: results are shown for the sequential treatment schedule for the Ovarian cancer cell line KURAMOCHI (left) and the bladder cancer cell line J82 (right). Two matrices are presented for each combination: the observed data surface on the left and the Loewe Excess on the right. The Synergy Score is denoted below each pair of matrices.

DETAILED DESCRIPTION OF THE INVENTION

[0018] Disclosed herein are methods of inhibiting tumor growth in a subject, *e.g.*, a human, by administration of a combination of a Chk1 inhibitor (*e.g.*, SRA737) and a Wee1 inhibitor to the subject. Also disclosed herein are combinations of a Chk1 inhibitor and a Wee1 inhibitor in, *e.g.*, pharmaceutical compositions and/or kits, useful for practicing the methods of inhibiting tumor growth. Also disclosed herein are methods of reducing expression of a marker gene in a cell, of inhibiting a Wee1 in a cell, and of activating a CDC25 and CDK1/2 in a cell, by administration of a combination of a Chk1 inhibitor and a Wee1 inhibitor to the cell, either *in vivo* or *in vitro*.

[0019] Described herein is the surprising result that pharmacological inhibition of Wee1 kinase activity potentiates the efficacy of SRA737 when administered in accordance with the methods of the invention. The combination of SRA737 and a Wee1 inhibitor displays unexpected and synergistic effects useful for inhibiting tumor growth and for treating disorders such as cancer.

[0020] As described in detail below, *in vitro* screening assays were performed with the ATPlite endpoint and demonstrated the synergistic anti-tumor activity of the combination of SRA737 and a Wee1 inhibitor. SRA737 activity was significantly enhanced by a Wee1 inhibitor in SW620, HT-29, Calu-6, KURAMOCHI, OVCAR-3, 5673, TCCSUP, J82, MIA PaCa-2, Panc 03.27, FaDu and CAL-27 cancer cell lines. In contrast, SRA737 activity was not affected by the presence of additional Wee1 inhibitors in the wild-type cell lines. Finally, Wee1inhibitors such as MK-1775, in combination with SRA737 demonstrated significantly increased anti-cancer efficacy across a broad range of cell line panels representative of tumor types that include, but certainly are not limited to: bladder, colorectal, lung, ovary, pancreas and head and neck cancer cell lines.

[0021] Apropos, the Chk1 inhibitor and a Wee1 inhibitor combination is significantly more potent and effective for inhibiting tumor growth, for inhibiting cancer cell replication, *e.g.*, inhibiting the growth of tumor cells, inhibiting Chk1, CDK1/2 and/or CDC25 kinase activity than either a Chk1 inhibitor or a Wee1 inhibitor alone.

Definitions

[0022] Terms used in the claims and specification are defined as set forth below unless otherwise specified.

[0023] The practice of the present invention includes the use of conventional techniques of organic chemistry, molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art.

[0024] In this application, reference will be made to a number of technical designations. All numerical designations, *e.g.*, pH, temperature, time, concentration, and weight, including ranges of each thereof, are approximations that typically may be varied (+) or (-) by increments of 0.1, 1.0, or 10.0, as appropriate. Reagents described herein are exemplary and equivalents of such may be known in the art.

[0025] Compounds utilized in the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers, regioisomers and individual isomers (e.g., separate enantiomers) are all intended to be encompassed within the scope of the present invention. The compounds of the present invention may also contain

unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example, and without limitation, tritium (3H), iodine-125 (125I), or carbon-14 (14C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

[0026] The term “subject” refers to any mammal including humans, and so includes mammals such as those animals of veterinary and research interest that are including, but not limited to: simians, cattle, horses, dogs, cats, and rodents with a tumor or cancer.

[0027] The term "administering" or "administration” of' a drug and/or therapy to a subject (and grammatical equivalents of this phrase) refers to both direct or indirect administration, which may be administration to a subject by a medical professional, may be self-administration, and/or indirect administration, which may be the act of prescribing or inducing one to prescribe a drug and/or therapy to a subject.

[0028] The term “treating” or “treatment of” a disorder or disease refers to taking steps to alleviate the symptoms of the disorder or disease, or otherwise obtain some beneficial or desired results for a subject, including clinical results. Any beneficial or desired clinical results may include, but are not limited to, alleviation or amelioration of one or more symptoms of cancer or conditional survival and reduction of tumor load or tumor volume; diminishment of the extent of the disease; delay or slowing of the tumor progression or disease progression; amelioration, palliation, or stabilization of the tumor and/or the disease state; or other beneficial results.

[0029] The term “*in situ*” or “*in vitro*” refers to processes that occur in a living cell growing separate from a living organism, *e.g.*, growing in tissue culture.

[0030] The term “*in vivo*” refers to processes that occur in a living organism.

[0031] The term “Chkl” or “CHEK1” or “Checkpoint kinase 1” refers to serine/threonine-protein kinase that is encoded by the CHEKl gene. Chk1 can also be referred to as Cell Cycle Checkpoint Kinase, CHK1 Checkpoint Homolog, EC 2.7.11.1 and EC 2.7.11. Chk1 refers to all alternatively spliced analogues and comprises Homo sapiens Chk1 isoforms encoded by amino acid sequences and nucleotide sequences according to National Center for Biotechnology Information (NCBI) accession numbers: NP_001107594.1, NP_001107593.1, NP_001265.2,

NP_001231775.1, NP_001317356.1, NP_001317357.1, XP_016872635.1, XP_024304105.1, and XP_011540862.1, NM_001114122, NM_001114121.2, NM_001274.5, NM_001244846.1, NM_001330427.1, NM_001330428.1, and XM_017017146.2.

[0032] The term "CDC25" refers to the dual phosphatase (in a sub-class of the protein tyrosine phosphatases) that dephosphorylates and concomitantly activates cyclin dependent kinases. CDC25 includes CDC25A, CDC25B, CDC25C and any alternatively spliced analogues. CDC25A can also be referred to as Cell Division Cycle 25A, Dual Specificity Phosphatase CDC25A, and M-Phase Inducer Phosphatase 1. CDC25A refers to all alternatively spliced analogues and comprises Homo sapiens CDC25A isoforms encoded by amino acid sequences and nucleotide sequences according to National Center for Biotechnology Information (NCBI) accession numbers: NP_001780 and NM_001789.2. CDC25B can also be referred to as: Cell Division Cycle 25B, M-Phase Inducer Phosphatase 2, and Dual Specificity Phosphatase Cdc25B. CDC25B refers to all alternatively spliced analogues and comprises Homo sapiens CDC25B isoforms encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_068659 and NM_021873.3. CDC25C can also be referred to as: Cell Division Cycle 25C, M-Phase Inducer Phosphatase 3, Dual Specificity Phosphatase Cdc25C, Protein Phosphatase 1, Regulatory Subunit 60, Mitosis Inducer CDC25 and PPP1R60. CDC25C refers to all alternatively spliced analogues and comprises Homo sapiens CDC25C isoforms encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_001781 and NM_001790.4.

[0033] The term "Wee1" refers to WEE1A Kinase. Wee1 can also be referred to as: WEE1, WEE1G2 Checkpoint Kinase, WEE1hu, WEE1A, Wee1-Like Protein Kinase and WEE1 Homolog. WEE1 refers to all alternatively spliced analogues and comprises Homo sapiens WEE1 isoforms encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_003381 and NM_003390.3.

[0034] The term "Chk1 inhibitor" refers to an inhibitor of Chk1 or CHEK1. A Chk1 inhibitor may be a small molecule, an antibody or a nucleic acid. A Chk1 inhibitor can reduce the expression of CHEK1, inhibit the activity or function of Chk1 in cells, or combinations thereof. Chk1 inhibitors include, but are not limited to: SRA737, Prexasertib (LY2606368) (Commercially available from Sellechchem, Catalog No. S7178), PF-477736 (Commercially

available from Sellechchem, Catalog No. S2904), AZD7762 (Commercially available from Sellechchem, Catalog No. S1532), Rabusertib (LY2603618) (Commercially available from Sellechchem, Catalog No. S2626), MK-8776 (SCH 900776) (Commercially available from Sellechchem, Catalog No. S2735), CHIR-124 (Commercially available from Sellechchem, Catalog No. S2683), SAR-020106 (Commercially available from Sellechchem, Catalog No. S7740) and CCT245737 (Commercially available from Sellechchem, Catalog No. S8253).

[0035] The term "Wee1 inhibitor" refers to an inhibitor of Wee1. A Wee1 inhibitor may be a small molecule, an antibody or a nucleic acid. A Wee1 inhibitor can reduce the expression of Wee1, inhibit the activity or function of Wee1 in cells, or combinations thereof. Wee1 inhibitors include, but are not limited to: MK 1775, PD166285, PF00120130, 4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3-(2H,6H)-dione (PHCD), ADC-730, ADC-999, and PD0407824.

[0036] The term "tumor suppressor gene" refers to any gene that increases a "hallmark of tumor growth or cancer" when inhibited, deleted, reduced in expression, or otherwise has reduced function in a cell. "Hallmarks of tumor growth or cancer" include, but are not limited to, sustained or increased proliferation of a cell, sustained or increased proliferative signaling in a cell, replicative immortality, resisting cell death (*e.g.*, apoptosis), evasion of growth suppression, avoidance of immune destruction, induction of angiogenesis, activation of invasive or metastatic potential, promotion of inflammation, deregulated cellular energetics, genome instability, and combinations thereof. Tumor suppressor genes include, but are not limited to, the following genes: CDKN1A, CDKN1B, CDKN2A, CDKN2B, CDKN2C, RB1, TP53, and any alternatively spliced analogues. CDKN1A can also be referred to as: Cyclin Dependent Kinase Inhibitor 1A, CDK-interacting Protein 1, CDKN1, CAP20, MDA-6, CIP1, SDI1, WAF1 and P21. CDKN1B can also be referred to as: Cyclin Dependent Kinase 1B, P27KIP1, KIP1, Cyclin Dependent Kinase Inhibitor P27, CDKN4, MEN1B, and MEN4. CDKN2A can also be referred to as: Cyclin Dependent Kinase Inhibitor 2A, Cyclin-Dependent Kinase 4 Inhibitor A, Multiple Tumor Suppressor 1, P16-INK4A, P14ARF, CDKN2, CDK4I, MTS-1, MTS1 and MLM. CDKN2B can also be referred to as: Cyclin Dependent Kinase Inhibitor 2B, Cyclin-Dependent Kinase 4 Inhibitor B, Multiple Tumor Suppressor 2, P14-INK4b, P15-INK4b, MTS-2, MTS2, P14 CDK Inhibitor, P15 CDK Inhibitor, CDK4B inhibitor, INK4B, TP15 and P15. CDKN2C can also be referred to as: Cyclin Dependent Kinase Inhibitor 2C, P18-INK4C, P18-INK6,

Cyclin-Dependent Kinase 6 Inhibitor P18, Cyclin-Dependent Kinase 4 Inhibitor C, CDK6 inhibitor P18, CDKN6, INK4C and P18. RB1 can also be referred to as: RB, Retinoblastoma 1, Retinoblastoma-Associated Protein, RB Transcriptional Corepressor, Protein Phosphatase 1Regulatory Subunit 130, P105-Rb, Pp110, and PRb. TP53 can also be referred to as: P53, Tumor Protein 53, Phosphoprotein P53, P53 Tumor Suppressor, Tumor Suppressor P53, TRP53, Antigen NY-CO-13, BCC7, and LFS1. CDKN1A comprises Homo sapiens CDKN1A encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_001207707 and NM_001220778.1. CDKN1B comprises Homo sapiens CDKN1B encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_004055 and NM_004064. CDKN2A comprises Homo sapiens CDKN2A encoded by amino acid sequences and nucleotide sequences according to National Center for Biotechnology Information (NCBI) accession numbers: NP_478102 and NM_058195.3. CDKN2B comprises Homo sapiens CDKN2B encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_004927 and NM_004936.3. CDKN2C comprises Homo sapiens CDKN2C encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_001253 and NM_001262.2. RB1 comprises Homo sapiens RB1 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_000312 and NM_000321.2. TP53 comprises Homo sapiens TP53 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_000537 and NM_000546.5.

[0037] The term “DNA damage repair (DDR) gene” or “DNA damage repair pathway gene” refers to any gene that directly or indirectly promotes repair of DNA mutations, breaks or other DNA damage or structural changes. DNA damage repair (DDR) genes include, but are not limited to, the following genes: ATM, BLM, BRCA1, BRCA2, CHEK2, MLH1, MSH2, PALB2, POLD1, RAD50, RAD51, RAD51B, RAD51C, RAD51D, MSH6, PMS2, RAD52, RAD54L, RPA1, SETD2, SMARCA4, TP53BP1, XRCC2, XRCC3, POLE, KMT2D and ARID1A. DDR genes also include genes in the Fanconi anemia (FA) pathway. Genes in the FA pathway include, but are not limited to, Fanconi anemia complementation group (FANC) genes, such as FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM and any alternatively spliced analogues. ATM can also be referred to as: ATM Serine/Threonine Kinase, Ataxia Telangiectasia Mutated, A-T mutated, TELO1, TEL1, ATDC, AT1, ATE, ATA,

ATC and ATD. BLM can also be referred to as: Bloom Syndrome RecQ Like Helicase, DNA Helicase RecQ-like Type 2, RecQ Protein-like 3, RECQL3, RECQL2, RECQ2, and Bloom Syndrome Protein. BRCA1 is also referred to as: BRCA/BRCA1-Contining Complex Subunit 1, Protein Phosphatase 1 Regulatory Subunit 53, Fanconi Anemia Complementation Group S, RING Finger Protein 53, BROVCA1, PPP1R53, BRCAI and BRCC1. BRCA2 can also be referred to as: BRCA/BRCA1-Contining Complex Subunit 2, Fanconi Anemia Group D1 Protein, Fanconi Anemia complementation Group D1, Breast Cancer 2 Tumor Suppressor, Breast and Ovarian Cancer Susceptibility Protein 2, FANCD1, FACD, FANCD, FAD1, GLM3 and FAD. CHEK2 is also referred to as: Checkpoint Kinase 2, CHK2 Checkpoint Homolog, CHK2, CDS1, Cds1 Homolog, HCds1, RAD53, PP1425 and LFS2. MLH1 can also be referred to as: MutL Homolog 1, DNA Mismatch Repair Protein Mlh1, COCA2, HNPCC, HNPCC, HMLH1 and FCC2. MSH2 can also be referred to as: MutS Homolog 2, HMSH2, DNA Mismatch Repair Protein Msh2, MutS Protein Homolog 2, HNPCC1, HNPCC, LCFS2, COCA1 and FCC1. PALB2 can also be referred to as: Partner and Localizer of BRACA2, FANCN, Fanconi Anemia Complementation Group N and PNCA3. POLD1 can also be referred to as DNA Polymerase Delta 1 Catalytic Subunit, DNA Polymerase Subunit Delta P125, PPOLD, CDC2 Homolog, CRCS10, CDC2 and MDPL. RAD50 can also be referred to as: RAD50 Double Strand Break Repair Protein, HRad50, DNA Repair Protein RAD50, RAD502 and NBSLD. RAD51 can also be referred to as: RAD51 Recombinase, RAD51 Homolog A, HRAD51, RAD51A, RECA, Rec-A like protein, Recombination Protein A, HsT16930, BRCC5, FANCR and MRMV2. RAD51B can also be referred to as: RAD51 Paralog B, RAD51 Homolog B, RAD51 Homolog 2, RAD51L1, REC2, DNA Repair Protein RAD51 Homolog B, RAD51-Like 1 and RAD51-Like Protein 1. RAD51C can be referred to as: RAD51 Paralog C, RAD51-Like Protein 2, RAD51 Homolog 3, RAD51L2, R51H2, DNA Repair Protein RAD51 Homolog 3, BROVCA3 and FANCO. RAD51D can also be referred to as: RAD51 Paralog D, RAD51 Homolog 4, RAD51-Like Protein 3, RAD51L3, R51H3, DNA Repair Protein RAD51 Homolog 4, TRAD and BROVCA4. MSH6 can also be referred to as: MutS Homolog 6, G/T Mismatch-Binding Protein, MutS Protein Homolog 6, DNA Mismatch Repair Protein Msh6, GTMBP, GTBP, P160, HNPCC5, HMSH6 and HSAP. PMS2 can also be referred to as: PMS1 Homolog 2 Mismatch Repair Protein, DNA Mismatch Repair Protein PMS2, PMS1 Protein Homolog 2, PMSL2, PMS2 Post-meiotic Segregation Increased 2, HNPCC4, PMS2CL and

MLH4. RAD52 can also be referred to as: RAD52 Homolog DNA Repair Protein. RAD54L can also be referred to as: RAD54 Homolog, RAD54 Like, RAD54A, HRAD54, HHR54, HR54 and DNA Repair and Recombination Protein RAD54-Like. RPA1 can also be referred to as: Replication Protein A1, Single-Stranded DNA-Binding Protein, Replication Factor A Protein 1, RF-A Protein 1, REPA1, RPA70, MSTP075, MST075, HSSB, RF-A and RP-A. SETD2 can also be referred to as: SET Domain Containing 2, Protein-Lysine N-Methyltransferase SETD2, Huntingtin-Interacting Protein B, Lysine N-Methyltransferase 3A, P231HBP, HIP-1, HIF-1, KMT3A, HYPB, SET2, Histone-Lysine N-Methyltransferase SETD2, Huntington Interacting Protein 1, SET Domain-Containing Protein 2, KIAA1732, HSPC069, HBP231, HSET2, HIF1 and LLS. SMARCA4 can also be referred to as: SWI/SNF Related Matrix Associated Actin Dependent Regulator of Chromatin Subfamily A Member 4, Mitotic Growth and Transcription Activator, ATP-Dependent Helicase SMARCA4, BRG-1 Associated Factor 190A, Protein Brahma Homolog 1, BRM/SWI2-Related Gene, Homeotic Gene Regulator, Brahma Protein-Like 1, Nuclear Protein GRB1, Protein BRG-1, SNF2-Like 4, SNF2-Beta, BAF 190A, BRG1, SNF2LB, BAF190, HSNF2b, MRD16, RTPS2, SNF2B, SWI2, SNF2 and CSS4. TP53BP1 can also be referred to as: Tumor Protein P53 Binding Protein 1, P53-Binding Protein 1, P53BP1, 53BP1, Tumor Suppressor P53-Binding Protein 1, Tumor Protein P53-Binding Protein 1, Tumor Protein 53-Binding Protein, TP53-Binding Protein 1, TDRD30 and P202. XRCC2 can also be referred to as: X-RAY Repair Cross Complementing 2, X-Ray Repair Complementing Protein 2, DNA Repair Protein XRCC2, and FANCU. XRCC3 can also be referred to as: X-RAY Repair Cross Complementing 3, X-Ray Repair Complementing Protein 3, DNA Repair Protein XRCC3 and CMM6. POLE can also be referred to as: DNA Polymerase Epsilon Catalytic Subunit, DNA Polymerase Epsilon Catalytic Subunit A, DNA Polymerase II Subunit A, POLE1, CRCS12 and FILS. KMT2D can also be referred to as: MLL2, Lysine Methyltransferase 2D, Myeloid/Lymphoid or Mixed-Lineage Leukemia 2, Lysine-Specific Methyltransferase 2D, Lysine-N-Methyltransferase 2D, ALL-Related Protein, Histone-Lysine N-Methyltransferase 2D, Kabuki Mental Retardation Syndrome, MLL4, ALR, CAGL114, KABUK1, TNRC21, AAD10 and KMS. ARID1A can also be referred to as: AT-Rich Interaction Domain 1A, SWI/SNF-Related Matrix-Associated Actin Dependent Regulator of Chromatin Subfamily F Member 1, AT Rich Interactive Domain 1A, ARID Domain-Containing Protein 1A, SWI/SNF Complex Protein P270, BRG-1 Associated Factor 250a, BRG-1 Associated Factor 250, SWI-Like Protein,

Osa Homolog 1, BAF250a, SMARCF1, BAF250, HOSA1, B120, OSA1, Chromatin Remodeling Factor P250, BM029, MRD14, CSS2, P270 and ELD. ATM comprises Homo sapiens ATM encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_000042 and NM_000051.3. BLM comprises Homo sapiens BLM encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_000048 and NM_000057.3. BRCA1 comprises Homo sapiens BRCA1 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_009225 and NM_007294.3. BRCA2 comprises Homo sapiens BRCA2 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_000050 and NM_000059.3. CHEK2 comprises Homo sapiens CHEK2 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_009125 and NM_007194.3. MLH1 comprises Homo sapiens MLH1 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_000240 and NM_000249.3. MSH2 comprises Homo sapiens MSH2 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_000242 and NM_000251.2. PALB2 comprises Homo sapiens PALB2 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_078951 and NM_024675.3. POLD1 comprises Homo sapiens POLD1 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_002682 and NM_002691.3. RAD50 comprises Homo sapiens RAD50 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_005723 and NM_005732.3. RAD51 comprises Homo sapiens RAD51 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_002866 and NM_002875.4. RAD51B comprises Homo sapiens RAD51B encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_002868.1 and NM__002877.5. RAD51C comprises Homo sapiens RAD51C encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_478123 and NM_058216.2. RAD51D comprises Homo sapiens RAD51D encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_002869 and NM_002878.3. MSH6 comprises Homo sapiens MSH6 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_000170 and NM_000179.2. PMS2 comprises Homo sapiens PMS2 encoded by amino acid sequences and

nucleotide sequences according to NCBI accession numbers: NP_000526 and NM_000535.6. RAD52 comprises Homo sapiens RAD52 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_602296 and NM_134424.3. RAD54L comprises Homo sapiens RAD54L encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_003570 and NM_003579.3. RPA1 comprises Homo sapiens RPA1 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_002936 and NM_002945.4. SETD2 comprises Homo sapiens SETD2 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_054878 and NM_014159.6. SMARCA4 comprises Homo sapiens SMARCA4 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_003063, NM_003072.3, NP_001122321 and NM_001128849.1. TP53BP1comprises Homo sapiens TP53BP1 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_001135452 and NM_001141980.2. XRCC2 comprises Homo sapiens XRCC2 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_005422 and NM_005431.1. XRCC3 comprises Homo sapiens XRCC3 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_005423 and NM_005432.3. POLE comprises Homo sapiens POLE encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_006222 and NM_006231.3. KMT2D comprises Homo sapiens KMT2D encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_003473 and NM_003482.3. ARID1A comprises Homo sapiens ARID1A encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_006006 and NM_006015.5.

[0038] FANCA can also be referred to as: Fanconi Anemia Complementation Group A, FANCH, FACA, FAA, Fanconi Anemia Type 1, FA-H, FA1, FAH and FA. FANCC can also be referred to as: Fanconi Anemia Complementation Group C, FACC, FAC, Fanconi Anemia Group C Protein, and FA3. FANCD2 is also referred to as Fanconi Anemia Complementation Group D2, Fanconi Anemia Group D2 Protein, FANCD, FA-D2, FAD2, FA4. FANCE can also be referred to as: Fanconi Anemia Complementation Group E, Fanconi Anemia Group E Protein, FACE and FAE. FANCF can also referred be to as: Fanconi Anemia Complementation Group F, Fanconi Anemia Group F Protein, FACF and FAF. FANCG can also be referred to as:

Fanconi Anemia Complementation Group G, Fanconi Anemia Group G Protein, DNA Repair Protein XRCC9, XRCC9 Truncated Fanconi Anemia Group G Protein, FACG and FAG. FANCI can also be referred to as: Fanconi Anemia Complementation Group I, Fanconi Anemia Group I Protein, KIAA1794 and FACI. FANCL can also be referred to as: Fanconi Anemia Complementation Group L, Fanconi Anemia Group L Protein, RING-Type E3 Ubiquitin Transferase FANCL, PHD Finger Protein 9, FAAP43, PHF9, E3 Ubiquitin-Protein Ligase FANCL and POG. FANCM can also be referred to as: Fanconi Anemia Complementation Group M, Fanconi Anemia Group M Protein, ATP-Dependent RNA Helicase FANCM, KIAA1596, Protein Hef Ortholog, FAAP250 and Protein FACM. FANCA comprises Homo sapiens FANCA encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_000126 and NM_000135.3. FANCC comprises Homo sapiens FANCC encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_000127 and NM_000136.2. FANCD2 comprises Homo sapiens FANCD2 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_149075, NM_033084.4, NP_001306913 and NM_001319984.1. FANCE comprises Homo sapiens FANCE encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_068741 and NM_021922.2. FANCF comprises Homo sapiens FANCF encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_073562 and NM_022725.3. FANCG comprises Homo sapiens FANCG encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_004620 and NM_004629.1. FANCI comprises Homo sapiens FANCI encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_001106849 and NM_001113378.1. FANCL comprises Homo sapiens FANCL encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_001108108, NM_001114636.1, NP_060532 and NM_018062.3. FANCM comprises Homo sapiens FANCM encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_065988 and NM_020937.3.

[0039] The term “replication stress gene” refers to any gene that is induced or activated upon exposure of a cell increased DNA replication, increased initiation of replication (*i.e.*, entry into S phase of cell cycle) increased mitosis, increased cell proliferation, increased DNA damage, excessive compacting of chromatin, over-expression of oncogenes or combinations thereof, and

mediate the response to the stress, such as a stalled replication fork. Replication stress genes include, but are not limited to, the following genes: ATR, CHEK1 and any alternatively spliced analogues. ATR can also be referred to as: ATR Serine/Threonine Kinase, Ataxia Telangiectasia and RAD3-Related Protein, FRP1, MEC1 Mitosis Entry Checkpoint 1, FRAP Related Protein 1, FCTCS, SCKL1, MEC1 and SCKL. ATR comprises Homo sapiens ATR encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_001175 and NM_001184.3.

[0001] The term "oncogenic driver gene" or "oncogene" refers to any gene that when activated, over-expressed or otherwise increased in activity or abundance, leads to increased one or more hallmarks of tumor growth or cancer in a cell. Oncogenic driver genes include, but are not limited to, the following genes: CCNE1, MDM2, KRAS, MYC, MYCN, and any alternatively spliced analogues. In addition, negative regulators of oncogenic drivers, such as FBXW7, can also be viewed as oncogenic if mutation results in loss-of-function or reduced function. CCNE1 can also be referred to as: Cyclin E1, CCNE, G1/S-Specific Cyclin E1, Cyclin Es, Cyclin Et and PCCNE1. KRAS can also be referred to as KRAS Proto-Oncogene GTPase, V-Ki-Ras2 Kirsten Rat Sarcoma Viral Oncogene Homolog, V-Ki-Ras2 Kirsten Rat Sarcoma Viral Oncogene Homolog, Kirsten Rat Sarcoma Viral Proto-Oncogene, Cellular C-Ki-Ras2 Proto-Oncogene, Transforming Protein P21, C-Kirsten-Ras Protein, KRAS2A, K-RAS2B, K-RAS4A, K-RAs4B, K-Ras, KRAS1, C-Ki-Ras, K-Ras 2, C-K-RAS, CFC2, RALD, NS3 and NS. Myc can also be referred to as: C-Myc, MYC Proto-Oncogene BHLH Transcription Factor, V-Myc Avian Myelocytomatosis Viral Oncogene Homolog, Class E Basic Helix-Loop-Helix Protein 39, Proto-Oncogene C-Myc, BHLHe39, Avian Myelocytomatosis Viral Oncogene Homolog, Myc Proto-Oncogene Protein, MRTL and MYCC. MYCN can also be referred to as: N-MYC, MYCN Proto-Oncogene BHLH Transcription Factor, V-Myc Avian Myelocytomatosis Viral Oncogene Neuroblastoma Derived Homolog, Class E Basic Helix-Loop-Helix Protein 37, BHLHe37, NMYC, Neuroblastoma-Derived V-Myc Avian Myelocytomatosis Viral Related Oncogene, N-Myc Proto-Oncogene Protein, Neuroblastoma Myc Oncogene, Oncogene NMYC and ODED. MDM2 can also be referred to as: MDM2 Proto-Oncogene, MDM2 Proto-Oncogene E3 Ubiquitin Protein Ligase, Oncoprotein Mdm2, Hdm2, Mdm2 Transformed 3T3 Cell Double Minute 2 P53 Binding Protein, Double Minute 2 Human Homolog of P53-Binding Protein, RING-Type E3 Ubiquitin Transferase Mdm2, P53-Binding Protein Mdm2, Double Minute 2

Protein, ACTFS and HDMX. FBXW7 can also be referred to as: F-Box and WD Repeat Domain Containing 7, F-Box and WD Repeat Domain Containing 7, E3 Ubiquitin Protein Ligase, F-BOX Protein FBX30, Fbx30, SEL-10, SEL10, HCdc4, FBW7, HAgo, Archipelago Homolog, F-Box Protein SEL-10, Archipelago, FBXO30, FBXW6, CDC4, FBW6 and AGO. CCNE1 comprises Homo sapiens CCNE1 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_001229 and NM_001238.3. KRAS comprises Homo sapiens KRAS encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_203524 and NM_033360.3. MYC comprises Homo sapiens MYC encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_002458, NM_002467.5 and ABW69847. MYCN comprises Homo sapiens MYCN encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_005369 and NM_005378.5. MDM2 comprises Homo sapiens MDM2 encoded by amino acid sequences and nucleotide sequences according to NCBI accession numbers: NP_002383, NM_002392.5 and Q00987.

[0040] The term “percent identity,” in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (*e.g.*, BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the percent "identity" can exist over a region of the sequence being compared, *e.g.*, over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared. For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by

computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., infra). One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/). In some embodiments, polypeptide and nucleic acid sequences useful for the invention are at least 95, 96, 97, 98, or 99% identical to sequences described herein or referred to herein by a database accession number. In some embodiments, polypeptide and nucleic acid sequences useful for the invention are at least 95, 96, 97, 98, or 99% identical to any alternatively spliced analog sequences described herein or referred to herein by a database accession number. In some embodiments, polypeptide and nucleic acid sequences useful for the invention are 100% identical to sequences described herein or referred to herein by a database accession number

[0041] The term "effective amount" means an amount sufficient to produce a desired effect, *e.g.*, an amount sufficient to inhibit tumor growth.

[0042] The term "coadministration" contemplates that two or more compounds coadministered exert their pharmacological effect during the same period of time; such coadministration can be achieved by either simultaneous, contemporaneous, or sequential administration of the two or more compounds.

[0043] The term "QnD or qnd" refers to drug administration once every "n" days. For example, QD (or qd) refers to once every day or once daily dosing, Q2D (or q2d) refers to a dosing once every two days, Q7D refers to a dosing once every 7 days or once a week, Q5D refers to dosing once every 5 days, and so on.

[0044] The term "reduction" of a symptom or symptoms (and grammatical equivalents of this phrase) refers to decreasing the severity or frequency of the symptom(s), or elimination of the symptom(s).

[0045] It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[0046] Recitation of ranges herein includes the recited endpoints and all points there between.

Methods of the invention

[0047] Disclosed herein are methods of inhibiting tumor growth in a subject, *e.g.*, a human, by administration of both a Chk11 inhibitor and a Wee 1 inhibitor. A detailed description of the two compounds, kits comprising the compounds, and methods of use thereof are found below.

Tumor inhibition

[0048] The present disclosure is directed to methods using combinations of a Chk1 inhibitor (*e.g.*, SRA737) and a Wee1 inhibitor to inhibit the progression of, reduce the size in aggregation of, reduce the volume of, and/or otherwise inhibit the growth of a tumor. Also provided herein are methods of treating the underlying disease, *e.g.*, cancer, and extending the survival of the subject.

[0049] In one embodiment provided for is a method of inhibiting the growth of a tumor in a subject in need thereof, the method comprising administering to the subject a first effective amount of a Chk1 inhibitor and a second effective amount of a Wee1 inhibitor. In some aspects, the disclosure provides for a method of inhibiting the growth of a tumor, wherein tumor growth is reduced by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, 26%, 28%, 30%, 32%, 34%, 36%, 38%, 40%, 42%, 44%, 46%, 48%, 50%, 52%, 54%, 56%, 58%, 60%, 62%, 64%, 66%, 68%, 70%, 72%, 74%, 76%, 78%, 80%, 82%, 84%, 86%, 88%, 90%, 92%, 94%, 96%, 98%, or 100% as measured by tumor volume. In some aspects, the disclosure provides for a method of inhibiting the growth of a tumor, wherein tumor growth is reduced by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, 26%, 28%, 30%, 32%, 34%, 36%, 38%, 40%, 42%, 44%, 46%, 48%, 50%, 52%, 54%, 56%, 58%, 60%, 62%, 64%, 66%, 68%, 70%, 72%, 74%, 76%, 78%, 80%, 82%, 84%, 86%, 88%, 90%, 92%, 94%, 96%, 98%, or 100% as measured by the absolute size of the tumor. In some aspects, the disclosure provides for a method of inhibiting the growth of a tumor, wherein tumor growth is reduced by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, 26%, 28%, 30%, 32%, 34%, 36%, 38%, 40%, 42%, 44%, 46%, 48%,

50%, 52%, 54%, 56%, 58%, 60%, 62%, 64%, 66%, 68%, 70%, 72%, 74%, 76%, 78%, 80%, 82%, 84%, 86%, 88%, 90%, 92%, 94%, 96%, 98%, or 100% as measured by the expression levels of tumor markers for that type of tumor.

Additional methods

[0050] Further, the present disclosure provides a method of reducing the expression of a marker gene in a cell, the method comprising contacting the cell with a first effective amount of a Chk1 inhibitor (*e.g.*, SRA737) and a second effective amount of a Wee1 inhibitor. In some aspects, the disclosure provides for a method of reducing the expression of a marker gene in a cell, wherein the marker gene is selected from the group consisting of: a tumor suppressor gene, a DNA damage repair gene, a replication stress gene, and an oncogenic driver gene.

[0051] In some aspects, the disclosure provides for methods wherein the marker gene is MYC. In some aspects, the disclosure provides for methods wherein the marker gene is MYCN. In some aspects, the disclosure provides for methods wherein the marker gene is KRAS. In some aspects, the disclosure provides for methods wherein the marker gene is CHEK1. In some aspects, the disclosure provides for methods wherein the marker gene is CCNE1. In some aspects, the disclosure provides for methods wherein the marker gene is BRCA1. In some aspects, the disclosure provides for methods wherein the marker gene is BRCA2. In some aspects, the disclosure provides for methods wherein the marker gene is CHEK2.

[0052] Additionally, the present disclosure provides for a method of inhibiting Chk1 activity in a cell, the method comprising contacting the cell with a first effective amount of a Chk1 inhibitor (*e.g.*, SRA737) and a second effective amount of a Wee1 inhibitor. The present disclosure also provides for a method of inhibiting a CDC25 activity in a cell, the method comprising contacting the cell with a first effective amount of a Chk1 inhibitor and a second effective amount of a Wee1 inhibitor. The present disclosure also provides for a method of inhibiting a CDK1/2 activity in a cell, the method comprising contacting the cell with a first effective amount of a Chk1 inhibitor and a second effective amount of a Wee1 inhibitor. In some aspects, the disclosure provides for a method of inhibiting Chk1, CDK1/2, or CDC25 activity in a cell, wherein the first effective amount and second effective amount is an amount that produces an IC_{50} value of no more than 0.001 μM, no more than 0.01 μM, no more than 0.1 μM, no more than 1 μM, no more than 2 μM, no more than 3 μM, no more than 5 μM, no more than 6 μM, no

more than 8 μM, no more than 10μM, no more than 12 μM, no more than 14 μM, no more than 16 μM, no more than 18 μM, no more than 20 μM, no more than 25 μM, no more than 30 μM, no more than 35 μM, no more than 40 μM, no more than 50 μM, no more than 75 μM, or no more than 100 μM.

Effective amount

[0053] The present disclosure also provides for the combination of a Chk1 inhibitor (*e.g.*, SRA737) and a Wee1 inhibitor. The present disclosure further provides for the combination of a first effective amount of a Chk1 inhibitor and a second effective amount of a Wee1 inhibitor. The present disclosure further provides for a pharmaceutical composition comprising a first effective amount of a Chk1 inhibitor and a second effective amount of a Wee1 inhibitor and at least one pharmaceutically acceptable carrier or excipient. In some aspects, the present disclosure further provides for combinations where the first effective amount and the second effective amount are each an amount from about 0.001 mg/kg to about 15 mg/kg. In some embodiments the first effective amount of a Chk1 inhibitor and/or the second effective amount of a Wee1 inhibitor is 0.001, 0.005, 0.010, 0.020, 0.050, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 or 15.0 mg/kg.

Types of tumors

[0054] In some aspects, the present disclosure provides for methods of inhibiting the growth of a tumor wherein the tumor is from a cancer that is bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, mesothelioma, melanoma, myeloma, ovarian cancer, prostate cancer, pancreatic cancer, renal cell cancer, small cell lung cancer, or squamous cell carcinoma of the head and neck.

[0055] Accordingly, the present disclosure also provides for methods of treating a cancer in a subject in need thereof, the method comprising administering a first effective amount of a Chk1 inhibitor and a second effective amount of a Wee1 inhibitor to the subject. In some aspects, methods are disclosed for the treatment of cancer wherein the cancer is bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, mesothelioma, melanoma, myeloma, ovarian cancer, prostate cancer, pancreatic cancer, renal cell cancer, small cell lung cancer, or squamous cell carcinoma of the head and neck. In further aspects, methods of reducing expression of a marker

gene in a cell, methods of inhibiting Chk1, inhibiting CDK1/2 and/or methods of inhibiting CDC25 are provided for wherein the inhibition of Chk1, the inhibition of CDK1/2, the inhibition of CDC25, and/or the expression of a marker gene is in a cancer cell that is bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, mesothelioma, melanoma, myeloma, ovarian cancer, prostate cancer, pancreatic cancer, renal cell cancer, small cell lung cancer, or squamous cell carcinoma of the head and neck.

Cellular Assays

[0056] In some aspects, the present disclosure includes using assays to measure the anti-tumor and anti-cancer activity of the compounds alone, and then in combination. Briefly, cell lines were preserved in liquid nitrogen and thawed and expanded in growth media containing full serum. As the cells reached expected doubling times, the screening assay began. Cells were seeded in growth media and equilibrated via centrifugation. The cell cultures were placed in incubators and then underwent treatment. Assay plates were collected and ATP levels were measured. Assay plates were incubated with combinations for seventy two (72) or ninety six (96) hours in simultaneous and sequential dosing schedules.

[0057] In some aspects, the present disclosure involves using a compound library containing Wee1 compounds. As a control, SRA737 was tested alone to quantify its single agent activity against the same cancer lines, and then again in combination with Wee1 inhibitors so as to compare the synergistic activity of the two. A clustered analysis of their respective activity was correlated for an analysis of the target or mode-of-action. This analysis proved to be a useful method that results in an observed pattern of enhanced SRA737 activity of activity across the cell line panel.

[0058] The assays determine a comparative score based on the inhibition of cell growth as can be measured in numerous ways, for example ATP production or by MTT assay.

[0059] Potency shifting was evaluated using an isobologram, which demonstrates how much less drug is required in combination to achieve a desired effect level, when compared to the single agent doses needed to reach that effect. The isobologram was drawn by identifying the locus of concentrations that correspond to crossing the indicated inhibition level. This is done by finding the crossing point for each single agent concentration in a dose matrix across the

concentrations of the other single agent. Practically, each vertical concentration C_Y is held fixed while a bisection algorithm is used to identify the horizontal concentration C_X in combination with that vertical dose that gives the chosen effect level in the response surface $\boldsymbol{Z}(C_X,C_Y)$.. These concentrations are then connected by linear interpolation to generate the isobologram display. For synergistic interactions, the isobologram contour fall below the additivity threshold and approaches the origin, and an antagonistic interaction would lie above the additivity threshold. The error bars represent the uncertainty arising from the individual data points used to generate the isobologram. The uncertainty for each crossing point is estimated from the response errors using bisection to find the concentrations where $\boldsymbol{Z}-\sigma_Z(C_X,C_Y)$ and $\boldsymbol{Z}+\sigma_Z(C_X,C_Y)$ cross $\boldsymbol{I}_{cut}$, where σ_Z cross Icut, where σZ is the standard deviation of the residual error on the effect scale.

[0060] These assays were used to assess the combination of SRA737 and enhancers for synergy were performed in a panel of fifteen (15) human cancer cell lines. The goal of the screen was to identify powerful combinations that demonstrate selective, synergistic killing of tumor cells. SRA737 was combined with twenty four (24) compounds and tested across a cell line panel that included bladder, colorectal, lung, ovary, pancreas and head and neck cancer cell lines. The enhancer compounds chosen for inclusion in the combination with SRA737 were selected broadly amongst oncology therapeutics. The screen is also informative on the effects of dosing schedules for the compounds. Simultaneous dosing, as well as sequential dosing, was performed for each combination across the cell line panel. The screen used the ATPlite endpoint created forty eight (48) unique combinations.

Scoring

[0061] The results of the anti-tumor/ anti-cancer assay screen were analyzed and scores were developed to compare different combinations and assess the ability of each combination to surpass single agent activity.

[0062] Growth Inhibition (GI) as a measure of cell growth. GI percentages are calculated by applying the following test and equation:

$$\text{If } T < V_0 : 100 * \left(1 - \frac{T-V_0}{V_0}\right)$$

$$\text{If } T \geq V_0 : 100 * \left(1 - \frac{T-V_0}{V-V_0}\right)$$

where T is the signal measure for a test article at 72 or 96 hours, V is the untreated/vehicle-treated control measure, and V_0 is the untreated/vehicle control measure at time zero (also colloquially referred to as T_0 plates). This formula is derived from the Growth Inhibition calculation used in the National Cancer Institute's NCI-60 high throughput screen.

[0063] Also, inhibition is a measure of cell viability. Inhibition levels of 0% represent no inhibition of cancer cell growth and 100% represents no doubling of cell numbers during treatment. Inhibition Percentage is calculated as the following:

I=1-T/U

Where T is treated and U is untreated.

[0064] The scalar Synergy Score was devised to quantify synergistic interactions for the combinations. The Synergy Score is calculated as:

$$Synergy\ Score = \log f_X \log f_Y \sum \max(0, I_{data})\,(I_{data} - I_{Loewe})$$

Loewe Volume Score is calculated to quantify the magnitude of the combination activity in excess of additivity observed by a single agent. Additivity is calculated:

I_{Loewe} that satisfies $(X/X_I) + (Y/Y_I) = 1$

where X_I and Y_I are the single agent effective concentrations for the observed combination effect I.

[0065] In some aspects, the present disclosure provides methods wherein the clinical endpoint is identified by Synergy Score, Growth Inhibition Score or Loewe Volume Score scoring.

[0066] In one aspect, the endpoint is identified by Synergy Score as measured by the assays and formulas described herein.

[0067] In one aspect, the present disclosure provides for methods wherein the combination has a Loewe Volume score of no more than -40, -30, -20, -10, -5, or -1.

[0068] In one aspect, the present disclosure provides for methods wherein the combination has a Synergy Score of at least 2.8, 3.1, 3.5, 3.8, 4.2, 4.5, 5, 7, 10, 12, or 15.

In vitro methods

[0069] In one aspect, the present disclosure provides for methods for inhibiting the growth of a tumor by slowing or stopping cancerous tumor cells from replicating. Herein disclosed the combination of a Chk1 inhibitor and a Wee1 inhibitor are shown to arrest the reproduction of cancer cell lines indicative of bladder, colorectal, lung, ovary, pancreas and head and neck cancer cancers. Accordingly, provided for are methods for inhibiting the growth of a tumor, the method comprising contacting cancerous tumor cells with a combination of a Chk1 inhibitor and a Wee1 inhibitor, where the cancerous tumor cells include: bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, mesothelioma, melanoma, myeloma, ovarian cancer, prostate cancer, pancreatic cancer, renal cell cancer, small cell lung cancer, or squamous cell carcinoma of the head and neck cancerous cells.

[0070] Further, the present disclosure also provides methods of inhibiting Chk1 activity, inhibiting CDC25 activity, CDK1/2 activity and/or reducing expression of a marker gene in a cell, the method comprising contacting the cell with a first effective amount of a Chk1 inhibitor and a second effective amount of a Wee1 inhibitor. In each of these aspects, *i.e.,* for any and all such methods described herein foe methods of inhibiting tumor growth, inhibiting Chk1 activity, inhibiting CDK1/2 activity, inhibiting CDC25 activity, and/or activating cell cycle progression in a cell, the cell can be in a subject, but also the cell can be outside a biological context. And so methods are provided for *in vitro* where the cell is growing separate from a living organism, *e.g.,* growing in tissue culture. In the course of using said methods to inhibit the growth of tumor cells, it may be that some cells of the tumor or even cells in the microenvironment, or in the vicinity of the tumor, are not yet cancerous cells, but are pre-cancerous cells. Accordingly, the present disclosure provides for methods where the cell is cancerous and methods where the cell is not cancerous. With regard to methods *in vitro* where the cell is a cancer cell, the present disclosure provides for methods wherein the cancer is bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, mesothelioma, melanoma, myeloma, ovarian cancer, prostate cancer, pancreatic cancer, renal cell cancer, small cell lung cancer, or squamous cell carcinoma of the head and neck.

[0071] In an aspect, the present disclosure provides for methods wherein the cell is a MIA Pa Ca-2 pancreatic cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is a SW620 cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is a HT-29 cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is a Calu-6 cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is a NCI-H520 cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is an OV-90 cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is a KURAMOCHI cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is an OVCAR-3 cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is a 5637 cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is a TCCSUP cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is a J82 cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is a SNU-324 cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is a Panc 03.27 cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is a Cal-27 cancer cell. In some aspects, the present disclosure provides for methods wherein the cell is a FaDu cancer cell.

[0072] In an aspect, the present disclosure provides for methods wherein the cell is from a subject after administration of a combination as described herein. In one aspect, the present disclosure provides for methods wherein the cell is from a subject that has not been administered any anti-cancer treatment for at least one day, one week, one month, two months, three months, six months, or one year. In one aspect, the present disclosure provides for methods wherein the cell is from a human subject that has not been administered any anti-cancer treatment for at least one day, one week, one month, two months, three months, six months, or one year.

[0073] In some aspects, the present disclosure provides for methods that are performed *in vivo*. In some aspects, the present disclosure provides for methods that are performed *in vivo* with a human subject.

Clinical Endpoints

[0074] Provided herein are methods for inhibiting the growth of a tumor in a subject and/or cell, wherein the conditions of said methods are such that the method results in a clinically relevant endpoint.

[0075] Tumor growth occurs when one or more biological cells grow and divide much more rapidly resulting in an increase in the number of cells in comparison to the normal and healthy process of cells division. This phenomenon is an indication that the cells are in a disease state such as cancer or pre-cancer. Moreover, tumor growth oftentimes comes about in discrete stages prior to the agglomerated cells forming a tumor.

[0076] There are several methods the skilled artisan can use to measure cell replication rates. The overall metabolic activity inside a cell can be measured via a labeled biological product. For example, there are several commercially available dyes (*e.g.,* MTT) that can penetrate the cell and interact with certain enzymes and other factors to produce a detectable product. Also, cellular biomarkers can be measured in a cell. For example a BrdU assay can incorporate a thymidine derivative into cellular DNA and be detected with an antibody. Proliferating cell nuclear antigen (PCNA) is another such biomarker for detection. Besides tagging techniques, the skilled artisan can also use for example, microscopy or flow cytometry to allow for cell counts.

[0077] In one aspect, cellular replication is measured by a clinical endpoint that includes: a quality of life (QOL) score as determined from the disclosure herein, duration of response (DOR) score, as determined from the disclosure herein, clinical benefit rate (CBR) score, as determined from the disclosure herein, patient reported outcomes (PRO) score, as determined from the disclosure herein, an objective response rate (ORR) score as determined from the disclosure herein, a disease-free survival (DFS) or progression-free survival (PFS) score as determined from the disclosure herein, a time to progression (TTP) score as determined from the disclosure herein, an Overall Survival score as determined from the disclosure herein, a time-to-treatment failure (TTF) score as determined from the disclosure herein, RECIST criteria/ score as determined from the disclosure herein, and/or a Complete Response score as determined from the disclosure herein.

[0078] In some aspects, the present disclosure provides methods wherein the growth of the tumor is reduced no more than 5, 10, 20, 40, 50, 60, 80, 90, 95, 97, 99, or 99.9% after

administration of the first effective amount of SRA737, but before the administration of the second effective amount of a Wee1 inhibitor. In some aspects, the present disclosure provides methods wherein the growth of the tumor is reduced no more than 5, 10, 20, 40, 50, 60, 80, 90, 95, 97, 99, or 99.9% after administration of the second effective amount of a Wee1 inhibitor but before the administration of the first effective amount of SRA737.

[0079] In some aspects, the present disclosure provides methods wherein the % reduction is calculated based on measurement(s) of one or more clinical endpoints.

[0080] In some aspects, the present disclosure provides methods wherein the growth of the tumor is reduced as measured by an increase or a decrease in total cell count in a MTT assay, or by change in genetic profile as measured by a ctDNA assay, by no more than or at least 5, 10, 20, 40, 50, 60, 80, 90, 95, 97, 99, or 99.9% after administration of the first effective amount of SRA737, but before the administration of the second effective amount of a Wee1 inhibitor. In some aspects, the present disclosure provides methods wherein the growth of the tumor is reduced as measured by an increase or a decrease in total cell count in a MTT assay, or by change in genetic profile as measured by a ctDNA assay, by no more than or at least 5, 10, 20, 40, 50, 60, 80, 90, 95, 97, 99, or 99.9% after administration of the second effective amount of a Wee1 inhibitor but before the administration of the first effective amount of SRA737.

[0081] In some general aspects, the present disclosure provides methods wherein the growth of the tumor is reduced at least 5, 10, 20, 40, 50, 60, 80, 90, 95, 97, 99, or 99.9% after administration of the combination. In some aspects, the present disclosure provides methods wherein the growth of the tumor is reduced as measured by an increase or a decrease in total cell count in a MTT assay, or by change in genetic profile as measured by a ctDNA assay, by at least 5, 10, 20, 40, 50, 60, 80, 90, 95, 97, 99, or 99.9% after administration of the combination.

[0082] In some aspects, the present disclosure provides methods wherein administration results in an IC_{50} value below 10 μM and/or a GI_{50} value below 1 μM. In some aspects, the present disclosure provides methods wherein administration results in an IC_{50} value below 10 μM and/or a GI_{50} value below 1 μM at twenty-four (24) hours after administration. In some aspects, the present disclosure provides methods wherein administration results in an IC_{50} value below 10 μM and/or a GI_{50} value below 1 μM at forty-eight (48) hours after administration.

[0083] In some aspects, the present disclosure provides methods wherein the administration results in an AUC of at least 1, 10, 25, 50, 100, 200, 400, 600, 800, or 1000.

[0084] In some aspects, the present disclosure provides methods wherein the administration results in an IC_{50} value of no more than 0.001, 0.005, 0.01, 0.05, 0.1, 1, 3, 5, 10, 20, 40, 50, 60, 80, 90, 100, 200, 250, 300, 350, or 400 μM.

[0085] In some aspects, the present disclosure provides methods wherein the administration results in an EC_{50} value of at least 0.01, 0.1, 1, 3, 5, 10, 20, 40, 50, 60, 80, 90, 100, 200, 250, 300, 350, or 400 μM.

[0086] In some aspects, the present disclosure provides methods wherein the administration results in an therapeutic index (TI) value ranging from about 1.001:1 to about 50:1, about 1.1:1 to about 15:1, about 1.2:1 to about 12:1, about 1.2:1 to about 10:1, about 1.2:1 to about 5:1, or about 1.2:1 to about 3:1.

[0087] In some aspects, the present disclosure provides methods wherein the administration results in an GI_{50} value of at least 0.1 μM, 0.3 μM, 0.5 μM, 0.7 μM, 1 μM, 1.5 μM, 2 μM, 2.5 μM, 3 μM, 4 μM, 5 μM, or 10 μM.

[0088] In some aspects, the present disclosure provides methods wherein the administration results in a Maximum Response Observed (Max Response) value of no more than 0.1, 0.5, 1, 2 μM, 2.5 μM, 3 μM, 4 μM, 5 μM, or 10 μM.

[0089] Tumor growth can be expressed in terms of total tumor volume. There exist formulas, both generally speaking and specific to certain tumor models, that the skilled artisan can use to calculate tumor volume based upon the assumption that solid tumors are more or less spherical. In this regard, the skilled artisan can use experimental tools such as: ultrasound imaging, manual or digital calipers, ultrasonography, computed tomographic (CT), microCT, ^{18}F-FDG-microPET, or magnetic resonance imaging (MRI) to measure tumor volume. See for example Monga SP, Wadleigh R, Sharma A, et al. Intratumoral therapy of cisplatin/epinephrine injectable gel for palliation in patients with obstructive esophageal cancer. Am. J. Clin. Oncol. 2000;23(4):386–392; Mary M. Tomayko C., Patrick Reynolds, 1989. Determination of subcutaneous tumor size in athymic (nude) mice. Cancer Chemotherapy and Pharmacology, Volume 24, Issue 3, pp 148–154; E Richtig, G Langmann, K Müllner, G Richtig and J Smolle, 2004. Calculated tumour

volume as a prognostic parameter for survival in choroidal melanomas. Eye (2004) 18, 619–623; Jensen et al. BMC Medical Imaging 2008. 8:16; Tomayko et al. Cancer Chemotherapy and Pharmacology September 1989, Volume 24, Issue 3, pp 148–154; and Faustino-Rocha et al. Lab Anim (NY). 2013 Jun; 42(6):217-24, each of which are hereby incorporated by reference in their entirety.

[0090] In some aspects, the present disclosure provides methods wherein administration results in a reduction in tumor volume of at least 5, 10, 20, 40, 50, 60, 80, 90, 95, 97, 99 or 99.9% after administration of the combination. In some aspects, the present disclosure provides methods wherein administration results in a reduction in tumor size, as measured by medical imaging techniques, of at least 5, 10, 20, 40, 50, 60, 80, 90, 95, 97, 99 or 99.9% after administration of the combination.

[0091] In some aspects, the present disclosure provides methods wherein administration results in method where administration results in a reduction in tumor volume of at least 5% after one (1), two (2), three (3), four (4), six (6), eight (8), twelve (12), sixteen (16), twenty (20), twenty four (24), thirty six (36), or fifty two (52) weeks.

Indications and Genetic markers

[0092] Subjects with highest probability for effective tumor inhibition or cancer treatment include those which have cancer cells that carry mutations. Often tumor cells or otherwise cancer cells that are TP53-deficient display this kind of sensitivity. To increase the odds of successful treatment, scientists may determine the degree to which a tumor cell gene is mutated, the case of the former being the degree to which TP53 is mutated, or the degree to which the gene (TP53 former), or its penultimate gene product, is over-expressed (amplified) or under-expressed in the cancer cells, or inactivated via an amplification in MDM2. These scientific measurements are determined via methods well known to a skilled artisan, such as immunohistochemistry and reverse transcriptase polymerase chain reaction (RTPCR). For example, suitable methods for assessing p53 status are described in Chiaretti et al., 2011, Genes, Chrom. & Cancer 50: 263-274; and Berglind et al., 2008, Cancer Biol. & Ther. 7: 5, 699-708, each of which is incorporated herein by reference. Moreover, commercially available test kits, like the AmpliChip Test, are available to the skilled artisan for such purposes and do not take an overabundance of time or training to utilize.

[0093] The inventors observed that certain cancer cell lines, with pre-specified genetic mutations are 'more prone' to the strong combination activities of SRA737 and Wee1 inhibitors, demonstrating comparatively high Synergy Scores across the compound library.

[0094] In some aspects, the present disclosure provides for methods wherein the genetic marker is identified using a cancer cell that has a mutated gene for that marker. In some aspects, the genetic marker is identified by comparison with the genetic profile of non-cancerous cells, i.e., healthy cells. Apropos in some aspects, the present disclosure provides for methods wherein the genetic marker is identified using both cancer cells and non-cancer cells to determine one or more mutated genes, optionally where both the cancer cells and non-cancer cells are from a single human subject.

[0095] In some aspects, the present disclosure provides for methods of inhibiting the growth of a tumor where tumor cells have an identified genetic mutation when compared to the genetic sequence of matched control eukaryotic cells.

[0096] In some aspects, the present disclosure provides for methods wherein the tumor is a tumor from a cancer with a genetic mutation in a gene that is a tumor suppressor gene, a DNA damage repair gene, a replication stress gene, or an oncogenic driver gene and the cancer is bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, mesothelioma, melanoma, myeloma, ovarian cancer, prostate cancer, pancreatic cancer, renal cell cancer, small cell lung cancer, or squamous cell carcinoma of the head and neck.

[0097] In some aspects, the present disclosure provides for methods wherein the cancer is TP53 deficient cancer.

[0098] In some aspects, the present disclosure provides for methods wherein the subject is suffering from a tumor and/or cancer that has cells that overexpress the biomarker ORC1, CLSPN, or USP1. In some aspects, the present disclosure provides for methods wherein the subject is suffering from a tumor and/or cancer that has cells that under-express the marker gene RAD50.

[0099] In some aspects, the present disclosure provides for using high throughput genetic sequencing to select subjects suffering from cancer that have cells that provide a Synergy Score

above 2.5. In some aspects, the present disclosure provides for using high throughput genetic sequencing to select subjects suffering from cancer that have cells that provide a Loewe Volume Score above 8.0.

[00100] In some aspects, the present disclosure provides for 2D genetic screening of tumor cell lines and/or isogenic cell lines.

[00101] In some aspects, the present disclosure provides for methods wherein the genetic marker is identified by next generation sequencing.

[00102] In some aspects, the present disclosure provides for methods wherein the subject is screened to determine if their tumor and/or cancer provides a Synergy Score above 3.0 prior to administration of the combination of SRA737 and a Wee1 inhibitor. In some aspects, the present disclosure provides for methods wherein the subject is screened both before and after administration of the combination of SRA737 and a Wee1 inhibitor to determine one or more clinically relevant endpoints and to determine Synergy Score and/or Loewe Volume Score.

Subjects

[00103] The term "subject" is interchangeable with the term "patient." The present disclosure provides for administering the combination of a Chk1 inhibitor and a Wee1 inhibitor to a subject or a patient that is in need thereof. In some aspects, the tumor from a subject is screened with genetic testing and/sequencing prior to administration. In some aspects, the tumor from a subject is screened with genetic testing and/sequencing after administration. In some aspects, the tumor from a subject is screened both after and before administration. In some aspects, healthy cells from the subject are screened with genetic testing and/sequencing prior to administration, after administration, or both. In some aspects, the tumor from a subject is screened with other biological tests or assays to determine the level of expression of certain biomarkers. In some aspects, the tumor from a subject is screened with both genetic testing and/sequencing and other biomarker tests or assays.

[00104] In some aspects, the present disclosure provides for methods wherein the subject is a mammal. In some aspects, the present disclosure provides for methods wherein the subject is a primate.

[00105] In some aspects, the present disclosure provides for methods wherein the subject is a mouse.

[00106] In some aspects, the present disclosure provides for methods wherein the subject is a human.

[00107] In some aspects, the present disclosure provides for methods wherein the subject is a human that has a tumor having a genetic mutation in one or more of the following genes: a tumor suppressor gene, a DNA damage repair gene, a replication stress gene, or an oncogenic driver gene. In some aspects, the present disclosure provides for methods wherein the subject is a human that has a tumor that is TP53 deficient. In some aspects, the present disclosure provides for methods wherein the subject is a human that has a tumor that has a mutation in the CCNE1 gene. In some aspects, the present disclosure provides for methods wherein the subject is a human that has a tumor that has a mutation in the MYC gene. In some aspects, the present disclosure provides for methods wherein the subject is a human that has a tumor that has a mutation in the MYCN gene. In some aspects, the present disclosure provides for methods wherein the subject is a human that has a tumor that has a mutation in the CHEK1 gene. In some aspects, the present disclosure provides for methods wherein the subject is a human that has a tumor that has a mutation in the CHEK2 gene. In some aspects, the present disclosure provides for methods wherein the subject is a human that has a tumor that has a mutation in the KRAS gene.

[00108] In some aspects, the present disclosure provides for methods wherein the tumor is in a human suffering from cancer that is selected from the group consisting of: bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, mesothelioma, melanoma, myeloma, ovarian cancer, prostate cancer, pancreatic cancer, renal cell cancer, small cell lung cancer, and squamous cell carcinoma of the head and neck.

[00109] In some aspects, the present disclosure provides for methods wherein the subject is suffering from cancer in which the cancer cells have a genetic mutation in one or more of the following genes: a tumor suppressor gene, a DNA damage repair gene, a replication stress gene, or an oncogenic driver gene. In some aspects, the present disclosure provides for methods wherein the subject is a human suffering from cancer that is TP53 deficient cancer. In some

aspects, the present disclosure provides for methods wherein the subject is a human suffering from cancer that is a cancer with a mutation in the NRAS gene. In some aspects, the present disclosure provides for methods wherein the subject is a human suffering from cancer that is a cancer with a mutation in the BRCA1 gene.

[00110] In some aspects, the present disclosure provides for methods wherein the subject is a human suffering from cancer in which the cancer cells overexpress/ or under-express one or more biomarkers including: Myc, N-Myc, CCNE1, FBXW7, TP53, BRAC1 and Rb1.

Administration

[00111] As disclosed herein, the methods of the invention include coadministration of the combination of a Chk1 inhibitor (*e.g.*, SRA737) and a Wee1 inhibitor. Coadministered encompasses methods where a Chk1 inhibitor and a Wee1 inhibitor are given simultaneously, where a Chk1 inhibitor and a Wee1 inhibitor are given sequentially, and where either one of, or both of, a Chk1 inhibitor and a Wee1 inhibitor are given intermittently or continuously, or any combination of: simultaneously, sequentially, intermittently and/or continuously. The skilled artisan will recognize that intermittent administration is not necessarily the same as sequential because intermittent also includes a first administration of an agent and then another administration later in time of that very same agent. Moreover, the skilled artisan understands that intermittent administration also encompasses sequential administration in some aspects because intermittent administration does include interruption of the first administration of an agent with an administration of a different agent before the first agent is administered again. Further, the skilled artisan will also know that continuous administration can be accomplished by a number of routes including *i.v.* drip or feeding tubes, etc.

[00112] Furthermore, and in a more general way, the term "coadministered" encompasses any and all methods where the individual administration of a Chk1 inhibitor and the individual administration of a Wee1 inhibitor to a subject overlap during any timeframe.

[00113] The frequency of administration of a Chk1 inhibitor or a Wee1 inhibitor to a subject is known in the art as Qnd or qnd where n is the frequency in days for successive administration of that agent. For example, Q3d would be an administration of an agent once every three (3) days. Herein the present disclosure provides for methods comprising administering either one of, or both of, or any combinations thereof, a Chk1 inhibitor and/or a Wee1 inhibitor to a subject for

Q1d, Q2d, Q3d, Q4d, Q5d, Q6d, Q7d, Q8d, Q9d, Q10d, Q14d, Q21d, Q28d, Q30d, Q90d, Q120d, Q240d, or Q365d.

[00114] In an aspect, the present disclosure provides for methods where either one of or both of or any combination thereof a Chk1 inhibitor and/or a Wee1 inhibitor are administered intermittently. In one aspect, the present disclosure provides for methods comprising administering either one of, or both of, or any combinations thereof, a Chk1 inhibitor or a Wee1 inhibitor, to a subject with at least ten (10) minutes, fifteen (15) minutes, twenty (20) minutes, thirty (30) minutes, forty (40) minutes, sixty (60) minutes, two (2) hours, three (3) hour, four (4) hours, six (6) hours, eight (8) hours, ten (10) hours, twelve (12) hours, fourteen (14) hours, eighteen (18) hours, twenty-four (24) hours, thirty-six (36) hours, forty-eight (48) hours, three (3) days, four (4) days, five (5) days, six (6) days, seven (7) days, eight (8) days, nine (9) days, ten (10) days, eleven (11) days, twelve (12) days, thirteen (13) days, fourteen (14) days, three (3) weeks, or four (4) weeks, delay between administrations. In such aspects, the administration with a delay follows a pattern where ne of or both of or any combination thereof SRA737 and/or a Wee1 inhibitor are administered continuously for a given period of time from about ten (10) minutes to about three hundred and sixty five (365) days and then is not administered for a given period of time from about ten (10) minutes to about thirty (30) days. In one aspect, the present disclosure provides for methods where either one of or any combination of SRA737 and/or a Wee1 inhibitor are administered intermittently while the other is given continuously.

[00115] In an aspect, the present disclosure provides for methods where the combination of the first effective amount of the Chk1 inhibitor is administered sequentially with the second effective amount of a Wee1 inhibitor.

[00116] In an aspect, the present disclosure provides for methods where a Chk1 inhibitor and a Wee1 inhibitor are administered simultaneously. In one aspect, the present disclosure provides for methods where the combination of the first effective amount of a Chk1 inhibitor is administered sequentially with the second effective amount of a Wee1 inhibitor. In such aspects, the combination is also said to be "coadministered" since the term includes any and all methods where the subject is exposed to both components in the combination. However, such aspects are not limited to the combination being given just in one formulation or composition. It may be that certain concentrations of the Chk1 inhibitor and the Wee1 inhibitor are more advantageous to

deliver at certain intervals and as such, the first effective amount and second effective amount may change according to the formulation being administered.

[00117] In some aspects, the present disclosure provides for methods where the Chk1 inhibitor and the Wee1 inhibitor are administered simultaneously or sequentially. In some aspects, the present disclosure provides for methods where the first effective amount of the Chk1 inhibitor is administered sequentially after the second effective amount of a Wee1 inhibitor. In some aspects, the present disclosure provides for methods where the second effective amount of a Wee1 inhibitor is administered sequentially after the first effective amount of the Chk1 inhibitor.

[00118] In some aspects, the present disclosure provides for methods where the combination of a Chk1 inhibitor (*e.g.*, SRA737) and a Wee1 inhibitor is administered in one formulation. In some aspects, the present disclosure provides for methods where the combination is administered in two (2) compositions where the first effective amount of a Chk1 inhibitor is administered in a separate formulation from the formulation of the second effective amount of a Wee1 inhibitor.

[00119] In some aspects, the present disclosure provides for methods where the first effective amount of a Chk1 inhibitor is administered sequentially after the second effective amount of a Wee1 inhibitor. In some aspects, the present disclosure provides for methods where the second effective amount of a Wee1 inhibitor is administered sequentially after the first effective amount of a Chk1 inhibitor.

[00120] In some aspects, the present disclosure provides for methods where the first effective amount of a Chk1 inhibitor is administered no less than four (4) hours after the second effective amount of a Wee1 inhibitor. In one aspect, the present disclosure provides for methods where the first effective amount of a Chk1 inhibitor is administered no less than ten (10) minutes, no less than fifteen (15) minutes, no less than twenty (20) minutes, no less than thirty (30) minutes, no less than forty (40) minutes, no less than sixty (60) minutes, no less than one (1) hour, no less than two (2) hours, no less than four (4) hours, no less than six (6) hours, no less than eight (8) hours, no less than ten (10) hours, no less than twelve (12) hours, no less than twenty four (24) hours, no less than two (2) days, no less than four (4) days, no less than six (6) days, no less than eight (8) days, no less than ten (10) days, no less than twelve (12) days, no less than fourteen (14) days, no less than twenty one (21) days, or no less than thirty (30) days after the second

effective amount of a Wee1 inhibitor. In one aspect, the present disclosure provides for methods where the second effective amount of a Wee1 inhibitor is administered no less than ten (10) minutes, no less than fifteen (15) minutes, no less than twenty (20) minutes, no less than thirty (30) minutes, no less than forty (40) minutes, no less than sixty (60) minutes, no less than one (1) hour, no less than two (2) hours, no less than four (4) hours, no less than six (6) hours, no less than eight (8) hours, no less than ten (10) hours, no less than twelve (12) hours, no less than twenty four (24) hours, no less than two (2) days, no less than four (4) days, no less than six (6) days, no less than eight (8) days, no less than ten (10) days, no less than twelve (12) days, no less than fourteen (14) days, no less than twenty one (21) days, or no less than thirty (30) days after the first effective amount of a Chk1 inhibitor.

[00121] In some aspects, the present disclosure provides for methods where either one of, or both of, or any combination thereof, a Chk1 inhibitor and/or a Wee1 inhibitor are administered by a route selected from the group consisting of: intravenous, subcutaneous, cutaneous, oral, intramuscular, and intraperitoneal. In some aspects, the present disclosure provides for methods where either one of, or both of, or any combination thereof, a Chk1 inhibitor and/or a Wee1 inhibitor are administered intravenously. In some aspects, the present disclosure provides for methods where either one of, or both of, or any combination thereof, a Chk1 inhibitor and/or a Wee1 inhibitor are administered orally.

[00122] It is understood by the skilled artisan that the unit dose forms of the present disclosure may be administered in the same or different physicals forms, *i.e.,* orally via capsules or tablets and/or by liquid via *i.v.* infusion, and so on. Moreover, the unit dose forms for each administration may differ by the particular route of administration. Several various dosage forms may exist for either one of, or both of, the combination of Chk1 inhibitors and Wee1 inhibitors. Because different medical conditions can warrant different routes of administration, the same components of the combination described herein may be exactly alike in composition and physical form and yet may need to be given in differing ways and perhaps at differing times to alleviate the condition. For example, a condition such as persistent nausea, especially with vomiting, can make it difficult to use an oral dosage form, and in such a case, it may be necessary to administer another unit dose form, perhaps even one identical to other dosage forms used previously or afterward, with an inhalation, buccal, sublingual, or suppository route instead or as well. The specific dosage form may be a requirement for certain combinations of SRA737

and Wee1 inhibitor, as there may be issues with various factors like chemical stability or pharmacokinetics.

Therapeutically effective amount and Unit dose form

[00123] The methods of the invention include administration of a first effective amount of a Chk1 inhibitor (*e.g.*, SRA737) and a second effective amount of the Wee1 inhibitor. The term "therapeutically effective amount" is refers to an amount that is effective to ameliorate a symptom of a disease, *e.g.*, an amount that is effective to inhibit the growth of a tumor.

[00124] A therapeutically effective amount can be the same or different than either one of, or both of, the first effective amount and the second effective amount. This is because the present disclosure provides that the methods, as described herein, are effective even where neither of the first or second effective amounts must be an amount that, alone, will ameliorate a symptom of a disease. However, the present disclosure does provide that a therapeutically effective amount of the combination must be provided, *i.e.*, the combination does at least affect a treatment of a symptom of a disease.

[00125] A unit dose form is a term that is generally understood by the skilled artisan. A unit dose forms is a pharmaceutical drug product that is marketed for a specific use. The drug product includes the active ingredient(s) and any inactive components, most often in the form of pharmaceutically acceptable carriers or excipients. It is understood that multiple unit dose forms are distinct drug products. Accordingly, one unit dose form may be *e.g.*, the combination of a Chk1 inhibitor and a Wee1 inhibitor of 250 mg at a certain ratio of each component, while another completely distinct unit dose form is *e.g.*, the combination of a Chk1 inhibitor and a Wee1 inhibitor of 750 mg at the same certain ratio of each component referred to above. So from one unit dose form to another, the first effective amount and the second effective amount may both remain the same. Of course when the either one of the first effective amount or the second effective amount changes, the unit dose form is distinct.

[00126] In some aspects, the first effective amount is unique to the Chk1 inhibitor compound, *i.e.*, it is different than the second effective amount. In some aspects, the first effective amount is an amount that is equivalent to a "therapeutically effective amount" or an amount that brings about a therapeutic and/or beneficial effect. In some aspects, the first effective amount is a "therapeutically effective amount." In some aspects, the second effective amount is a

"therapeutically effective amount." In some aspects, both the first and second effective amounts are not a "therapeutically effective amount." In some aspects, the second effective amount is unique to the Wee1 inhibitor compound, *i.e.,* the second effective amount is a different amount for different Wee1 inhibitor compounds. In some aspects, the second effective amount is not sensitive to the identity of the Wee1 inhibitor and is a given amount no matter which a Wee1 inhibitor are in the combination.

[00127] In some aspects, the Chk1 inhibitor and a Wee1 inhibitor combination is formulated in one (1) unit dose form. In some aspects, the same unit dose form is administered for at least four (4) hours, six (6) hours, eight (8) hours, twelve (12) hours, twenty four (24) hours, one (1) day, two (2) days, three (3) days, seven (7) days, ten (10) days, fourteen (14) days, twenty one (21) days, or thirty (30) days.

[00128] In some aspects, the Chk1 inhibitor and a Wee1 inhibitor combination is formulated in at least two (2) separately distinct unit dose forms. In some aspects, the first effective amount is different in the first unit dose form than in the second unit dose form. In some aspects, the first effective amount is the same in the first unit dose form as it is in the second unit dose form.

[00129] In some aspects, the first unit dose form is the same as the second unit dose form. In some aspects, the first unit dose form is the same as the second and third unit dose forms. In some aspects, the first unit dose form is the same as the second, third, and fourth unit dose forms.

Compounds of the invention

[00130] In one aspect, the present disclosure provides for the combination of the compound SRA737 and Wee1 inhibitor compound(s), and methods of use.

Chk1 inhibitors

[00131] Chk1 inhibitors include, but are not limited to: SRA737, Prexasertib (LY2606368) (Commercially available from Sellechchem, Catalog No. S7178), PF-477736 (Commercially available from Sellechchem, Catalog No. S2904), AZD7762 (Commercially available from Sellechchem, Catalog No. S1532), Rabusertib (LY2603618) (Commercially available from Sellechchem, Catalog No. S2626), MK-8776 (SCH 900776) (Commercially available from Sellechchem, Catalog No. S2735), CHIR-124 (Commercially available from Sellechchem,

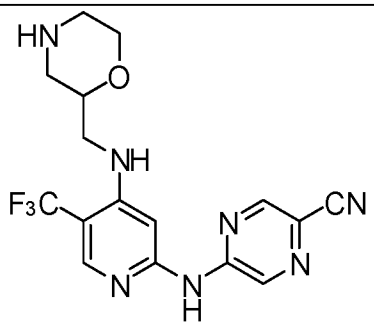
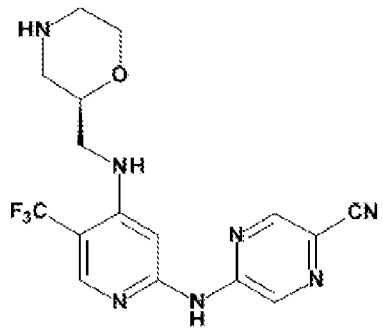
Catalog No. S2683), SAR-020106 (Commercially available from Sellechchem, Catalog No. S7740) and CCT245737 (Commercially available from Sellechchem, Catalog No. S8253).

SRA737

[00132] SRA737 is interchangeable with the terms "Sierra Compound 1" and "ProNAi Compound 1" as used herein. The compound SRA737 is also identified by the chemical name: 5-[[4-[[morpholin-2-yl]methylamino]-5-(trifluoromethyl)-2-pyridyl]amino]pyrazine-2-carbonitrile. Each of the enantiomers of SRA737 is useful for compositions, methods and kits disclosed herein.

[00133] SRA737 is a compound that is disclosed in international patent application no. PCT/GB2013/051233 and U.S. Patent No. 9,663,503, which are herein incorporated by reference in their entirety. The skilled artisan will find enabling methods for synthesizing SRA737 in international patent application no. PCT/GB2013/051233 and U.S. Patent No. 9,663,503. The synthesis of an enantiomer of SRA737 is found in the Examples section of PCT/GB2013/051233 on pages 40-42 (Syntheses 1A-1C).

[00134] In one aspect, the SRA737 structures are as shown in the table below.

Description	Structure
SRA737 structure	
enantiomer of SRA737	

Wee1 inhibitors

[00135] Wee1 is a nuclear kinase belonging to the serine/threonine family kinases. Wee1is known to regulate cell cycle progression as it influences cell size by inhibiting the entry into mitosis, through inhibition of Cdk1. Cell size is critical in the functionality of cells. Wee1 is known to determine the timing of entry of the cell into mitosis, producing smaller than normal daughter cell when inhibited. The inventors have found that Wee1 inhibitor compounds enhance the anti-tumor activity of SRA737. The Wee1 inhibitor compounds of the invention include any compound that has inhibits Wee1 activity and/or function and/or expression in cells (*e.g.*, tumor cells) *in vitro* and/or *in vivo*.

[00136] In an aspect, the wherein the Wee1 inhibitor is selected from the group consisting of: MK 1775, PD166285, PF00120130, 4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3-(2H,6H)-dione (PHCD), ADC-730, ADC-999, and PD0407824.

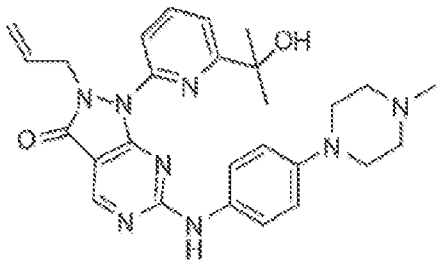
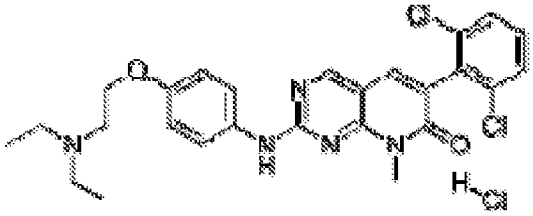
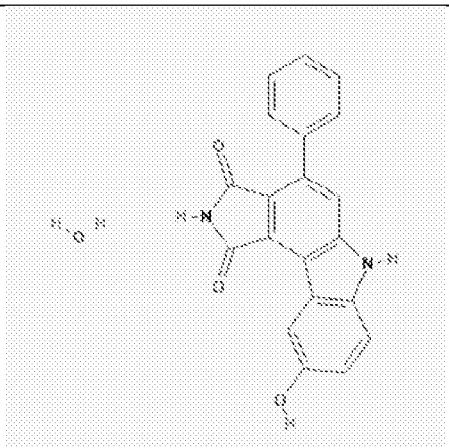
[00137] Wee1 inhibitors PF00120130 and MK-1775 are disclosed in international patent application no. PCT/US2011/060998, herein incorporated by reference, which provides general techniques known in the art to synthesize this compound and a variety of other heteroaryl Wee1 inhibitors. Wee1 inhibitor 4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3-(2H,6H)-dione (PHCD) is disclosed in Palmer et al. J. Med. Chem., 2006, 49 (16), pp 4896–4911, herein incorporated by reference. Sigma Aldrich commercially sells PHCD. Wee1 inhibitor PD0407824 and MK-1775 are disclosed in Ronco et al. Med. Chem. Commun., 2017, 8, 295-319, herein incorporated by reference, which provides general techniques known in the art to synthesize these compounds and a variety of other heteroaryl Wee1 inhibitors, such as ADC-730 and ADC-999, as referenced in Madhusudan, Srinivasan DNA Repair and Cancer: From Bench to Clinic CRC Press, Jan 22, 2013, herein incorporated by reference. Wee1 inhibitors ADC-730, ADC-239 and ADC-999 have chemical structures that are encompassed within, and the synthesis and use of said class of compounds is described in, international patent application nos. PCT/GB2014/051136 and PCT/GB2014/053793.

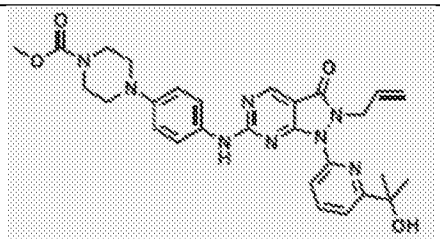
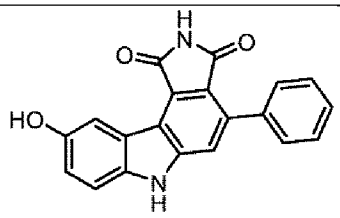
[00138] In general, Wee1 inhibitors, such as PF00120130, are compounds in which most often the core is a cyclic nitrogen-containing aromatic moiety and such compounds are known in the art, see for example and for the synthesis and use of said nitrogenous heteroaromatic compounds: International Publication WO 2010/098367, International Publication WO

2010/067886, International Publication WO 2008/115742, International Publication WO 2008/115738, International Publication WO 2007/126122, International Publication WO 2007/126128, International Publication WO 2004/007499 and United States Patent Application Publication 2005/0037476.

[00139] In an aspect, the Wee1 inhibitor is PD0407824. In an aspect, the Wee1 inhibitor is PHCD. In an aspect, the Wee1 inhibitor is PD166285. In an aspect, the Wee1 inhibitor is MK-1775. In an aspect, the Wee1 inhibitor is also an inhibitor of Chk1 enzyme activity.

[00140] In an aspect, the Wee1 inhibitor structures are as shown in the table below.

MK-1775 (Wee1 inhibitor)	
PD166285	
4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3-(2H,6H)-dione (PHCD)	

CJM061	
ADC-730	See international patent application no. PCT/GB2014/051136 or PCT/GB2014/053793
ADC-293	See international patent application no. PCT/GB2014/051136 or PCT/GB2014/053793
ADC-999	See international patent application no. PCT/GB2014/051136 or PCT/GB2014/053793
PD0407824	 PD407824

Pharmaceutical compositions of the invention

[00141] Methods for inhibiting the growth of a tumor, inhibiting the progression of or treating cancer are described herein. Said methods of the invention include administering a therapeutically effective amount or first effective amount of a Chk1 inhibitor (*e.g.*, SRA737) and therapeutically effective amount or second effective amount of a Wee1 inhibitor. The Chk1 inhibitor and the Wee1 inhibitor can each be formulated in pharmaceutical compositions. These pharmaceutical compositions may comprise, in addition to the active compound(s), a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material can depend on the route of administration, *e.g.*, oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

[00142] Pharmaceutical compositions for oral administration can be in tablet, capsule, powder or liquid form. A tablet can include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol can be included.

[00143] For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives can be included, as required.

[00144] A composition can be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

[00145] In general, the compounds of the present technology will be administered in a therapeutically effective amount by any of the accepted modes of administration for agents that serve similar utilities. The actual amount of the compound of the present technology, *i.e.*, the active ingredient, will depend upon numerous factors such as the severity of the disease to be treated, the age and relative health of the subject, the potency of the compound used, the route and form of administration, and other factors well known to the skilled artisan. The drug can be administered at least once a day, preferably once or twice a day.

[00146] An effective amount of such agents can readily be determined by routine experimentation, as can the most effective and convenient route of administration and the most appropriate formulation. Various formulations and drug delivery systems are available in the art. See, *e.g.*, Gennaro, A.R., ed. (1995) Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co.

[00147] A therapeutically effective dose can be estimated initially using a variety of techniques well-known in the art. Initial doses used in animal studies may be based on effective concentrations established in cell culture assays. Dosage ranges appropriate for human subjects can be determined, for example, using data obtained from animal studies and cell culture assays.

[00148] An effective amount or a therapeutically effective amount or dose of an agent, *e.g.*, a compound of the present technology, refers to that amount of the agent or compound that results in amelioration of symptoms or a prolongation of survival in a subject. Toxicity and therapeutic efficacy of such molecules can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, by determining the LD50 (the dose lethal to 50 % of the population) and the ED50 (the dose therapeutically effective in 50 % of the population). The dose ratio of toxic to therapeutic effects is therapeutic index, which can be expressed as the ratio LD50/ ED50. Agents that exhibit high therapeutic indices are preferred.

[00149] The effective amount or therapeutically effective amount is the amount of the compound or pharmaceutical composition that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician. Dosages particularly fall within a range of circulating concentrations that includes the ED50 with little or no toxicity. Dosages may vary within this range depending upon the dosage form employed and/or the route of administration utilized. The exact formulation, route of administration, dosage, and dosage interval should be chosen according to methods known in the art, in view of the specifics of a subject's condition.

[00150] Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety that are sufficient to achieve the desired effects; *i.e.*, the minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from, for example, *in vitro* data and animal experiments. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

[00151] The amount of agent or composition administered may be dependent on a variety of factors, including the sex, age, and weight of the subject being treated, the severity of the affliction, the manner of administration, and the judgment of the prescribing physician.

[00152] The present technology is not limited to any particular composition or pharmaceutical carrier, as such may vary. In general, compounds of the present technology will be administered as pharmaceutical compositions by any one of the following routes: oral, systemic (*e.g.*, transdermal, intranasal or by suppository), or parenteral (*e.g.*, intramuscular, intravenous or

subcutaneous) administration. The preferred manner of administration is oral using a convenient daily dosage regimen that can be adjusted according to the degree of affliction. Compositions can take the form of tablets, pills, capsules, semisolids, powders, sustained release formulations, solutions, suspensions, elixirs, aerosols, or any other appropriate compositions. Another preferred manner for administering compounds of the present technology is inhalation.

[00153] The choice of formulation depends on various factors such as the mode of drug administration and bioavailability of the drug substance. For delivery via inhalation the compound can be formulated as liquid solution, suspensions, aerosol propellants or dry powder and loaded into a suitable dispenser for administration. There are several types of pharmaceutical inhalation devices-nebulizer inhalers, metered dose inhalers (MDI) and dry powder inhalers (DPI). Nebulizer devices produce a stream of high velocity air that causes therapeutic agents (which are formulated in a liquid form) to spray as a mist that is carried into the subject's respiratory tract. MDI's typically are formulation packaged with a compressed gas. Upon actuation, the device discharges a measured amount of therapeutic agent by compressed gas, thus affording a reliable method of administering a set amount of agent. DPI dispenses therapeutic agents in the form of a free flowing powder that can be dispersed in the subject's inspiratory air-stream during breathing by the device. In order to achieve a free flowing powder, therapeutic agent is formulated with an excipient such as lactose. A measured amount of therapeutic agent is stored in a capsule form and is dispensed with each actuation.

[00154] Pharmaceutical dosage forms of a compound of the present technology may be manufactured by any of the methods well-known in the art, such as, for example, by conventional mixing, sieving, dissolving, melting, granulating, dragee-making, tabletting, suspending, extruding, spray-drying, levigating, emulsifying, (nano/micro-) encapsulating, entrapping, or lyophilization processes. As noted above, the compositions of the present technology can include one or more physiologically acceptable inactive ingredients that facilitate processing of active molecules into preparations for pharmaceutical use.

[00155] Recently, pharmaceutical formulations have been developed especially for drugs that show poor bioavailability based upon the principle that bioavailability can be increased by increasing the surface area *i.e.*, decreasing particle size. For example, U.S. Pat. No. 4,107,288 describes a pharmaceutical formulation having particles in the size range from 10 to 1,000 nm in

which the active material is supported on a crosslinked matrix of macromolecules. U.S. Patent No. 5,145,684 describes the production of a pharmaceutical formulation in which the drug substance is pulverized to nanoparticles (average particle size of 400 nm) in the presence of a surface modifier and then dispersed in a liquid medium to give a pharmaceutical formulation that exhibits remarkably high bioavailability.

[00156] The compositions are comprised of in general, a compound of the present technology in combination with at least one pharmaceutically acceptable excipient. Acceptable excipients are non-toxic, aid administration, and do not adversely affect therapeutic benefit of the claimed compounds. Such excipient may be any solid, liquid, semisolid or, in the case of an aerosol composition, gaseous excipient that is generally available to one of skill in the art.

[00157] Solid pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk and the like. Liquid and semisolid excipients may be selected from glycerol, propylene glycol, water, ethanol and various oils, including those of petroleum, animal, vegetable or synthetic origin, *e.g.*, peanut oil, soybean oil, mineral oil, sesame oil, etc. Preferred liquid carriers, particularly for injectable solutions, include water, saline, aqueous dextrose, and glycols.

[00158] Compressed gases may be used to disperse a compound of the present technology in aerosol form. Inert gases suitable for this purpose are nitrogen, carbon dioxide, etc. Other suitable pharmaceutical excipients and their formulations are described in Remington's Pharmaceutical Sciences, edited by E. W. Martin (Mack Publishing Company, 18th ed., 1990).

[00159] In some embodiments, the pharmaceutical compositions include a pharmaceutically acceptable salt. The term "pharmaceutically acceptable salt" refers to salts derived from a variety of organic and inorganic counter ions well known in the art that include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, and tetraalkylammonium, and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, and oxalate. Suitable salts include those described in Stahl and Wermuth (Eds.), Handbook of Pharmaceutical Salts Properties, Selection, and Use; 2002.

[00160] The present compositions may, if desired, be presented in a pack or dispenser device containing one or more unit dosage forms containing the active ingredient. Such a pack or device may, for example, comprise metal or plastic foil, such as a blister pack, or glass, and rubber stoppers such as in vials. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the present technology formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

[00161] The amount of the compound in a formulation can vary within the full range employed by those skilled in the art. Typically, the formulation will contain, on a weight percent (wt %) basis, from about 0.01-99.99 wt % of a compound of the present technology based on the total formulation, with the balance being one or more suitable pharmaceutical excipients. Preferably, the compound is present at a level of about 1-80 wt %. Representative pharmaceutical formulations are described below.

Formulation Examples

[00162] The following are representative pharmaceutical formulations containing the SRA737 and a Wee1 inhibitor, either alone or in combination.

Formulation Example 1 -- Tablet formulation

[00163] The following ingredients are mixed intimately and pressed into single scored table

Ingredient	Quantity per tablet (mg)
compound of this the present technology	400
Cornstarch	50
croscarmellose sodium	25
Lactose	120
magnesium stearate	5

Formulation Example 2 -- Capsule formulation

[00164] The following ingredients are mixed intimately and loaded into a hard-shell gelatin capsule.

Ingredient	Quantity per tablet (mg)
compound of this the present technology	200

lactose, spray-dried	148
magnesium stearate	25

Formulation Example 3 -- Suspension formulation

[00165] The following ingredients are mixed to form a suspension for oral administration.

Ingredient	Quantity per tablet (mg)
compound of this the present technology	1000
fumaric acid	500
sodium chloride	2000
methyl paraben	150
propyl paraben	50
granulated sugar	25
sorbitol (70% solution)	13
Veegum K (Vanderbilt Co.)	1000
Flavoring	0.035 mL
Colorings	500
distilled water	q.s. to 100 mL

Formulation Example 4 -- Injectable formulation

[00166] The following ingredients are mixed to form an injectable formulation.

Ingredient	Quantity per tablet (mg)
compound of this the present technology	1000
sodium acetate buffer solution, 0.4 M	2 mL
HCl (1N) or NaOH (1N)	q.s. to suitable pH
water (distilled, sterile)	q.s. to 20 mL

Formulation Example 5 -- Suppository Formulation

[00167] A suppository of total weight 2.5 g is prepared by mixing the compound of the present technology with Witepsol® H-15 (triglycerides of saturated vegetable fatty acid; Riches-Nelson, Inc., New York), and has the following composition:

Ingredient	Quantity per tablet (mg)
compound of this the present technology	500
Witepsol® H-15	balance

[00168] A composition can be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Kits

[00169] The present disclosure also provides for a kit comprising the combination of a Chk1 inhibitor (*e.g.*, SRA737) and a Wee1 inhibitor and instructions for use. The present disclosure further provides for a kit comprising one or more pharmaceutical compositions where the pharmaceutical composition(s) comprise a Chk1 inhibitor and a Wee1 inhibitor, and instructions for use, optionally the combination includes at least one pharmaceutically acceptable carrier or excipient.

[00170] Individual components of the kit can be packaged in separate containers and, associated with such containers, can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale. The kit may optionally contain instructions or directions outlining the method of use or administration regimen for the antigen-binding construct.

[00171] In some aspects, the disclosure provides for a kit comprising a combination of a Chk1 inhibitor and a Wee1 inhibitor and at least one pharmaceutically acceptable carrier or excipient.

[00172] When one or more components of the kit are provided as solutions, for example an aqueous solution, or a sterile aqueous solution, the container means may itself be an inhalant, syringe, pipette, eye dropper, or other such like apparatus, from which the solution may be administered to a subject or applied to and mixed with the other components of the kit.

[00173] The components of the kit may also be provided in dried or lyophilized form and the kit can additionally contain a suitable solvent for reconstitution of the lyophilized components. Irrespective of the number or type of containers, the kits described herein also may comprise an instrument for assisting with the administration of the composition to a patient. Such an

instrument may be an inhalant, nasal spray device, syringe, pipette, forceps, measured spoon, eye dropper or similar medically approved delivery vehicle.

[00174] In another aspect described herein, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described herein, *e.g.*, inhibition of tumor growth is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, *iv.* solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container(s) holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the disorder and 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).

[00175] The article of manufacture in this embodiment described herein may further comprise a label or package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

EXAMPLES

[00176] Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

[00177] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, T.E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Company, 1993); A.L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition);

Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg *Advanced Organic Chemistry 3rd Ed.* (Plenum Press) Vols A and B(1992).

Methods

[00178] A high throughput screen for the inhibition of Chk1 with SRA737 in combination with Wee1 inhibitors is described. SRA737 is interchangeable with the term "ProNAi Compound 1" and "Sierra Compound 1" as used herein. Wee1 inhibitors are referred to as "partner compounds" and/or "enhancers" herein.

Anti-Proliferation Assay

[00179] Cell lines that have been preserved in liquid nitrogen were thawed and expanded in growth media containing full serum. Once cells have reached expected doubling times, screening began. Cells were seeded in growth media in black 384-well tissue culture treated plates at cell densities as listed in Appendix 1 incorporated into and part of this specification. Cells were equilibrated in assay plates via centrifugation and placed in incubators (attached to the Dosing Modules) at 37°C for 24 hours before treatment. At the time of treatment, a set of assay plates (which do not receive treatment) were collected and ATP levels were measured by adding ATPLite (Perkin Elmer). These Tzero (T0) plates were read using ultra-sensitive luminescence on Envision plate readers. Assay plates were incubated with compounds for 72 or 96 hours in two different dosing schedules and were then analyzed using ATPLite.

[00180] This combination screen included two different dosing schedules for ProNAi Compound 1 and the partner compound: co-treatment and sequential administration of ProNAi Compound 1 with each of the 24 partner compounds (Figure 1). In the co-treatment schedule, both enhancee (ProNAi Compound 1/ SRA737) and enhancer (partner compound) were added at time zero (0h). Cells were exposed to ProNAi Compound 1 (SRA737) and the enhancer for the entire 72-hour treatment time. In the sequential schedule, only the enhancer was added at time zero (0h) and ProNAi Compound 1 was added 24 hours after the addition of the enhancer. Cells were exposed to the enhancer for 96 hours, while the ProNAi Compound 1 treatment time was limited to the final 72 hours.

[00181] In Chalice Analyzer, the co-treatment and sequential dosing schedules are referred as the 72h and 96h treatment times, respectively. The sequential dosing schedule is also referred as the 0h/24h dose times. In the co-treatment schedule, both enhancee (ProNAi Compound 1/ SRA737) and enhancer (partner compound) were added at time zero. In the sequential treatment schedule, only the enhancer was added at time zero. The enhancee was added 24 hours after the addition of enhancer. All other assay conditions were identical in both dosing schedules.

[00182] All data points were collected via automated processes and were subject to quality control and analyzed using proprietary software. Assay plates were accepted if they pass the following quality control standards: relative raw values were consistent throughout the entire experiment, Z-factor scores were greater than 0.6 and untreated/vehicle controls behaved consistently on the plate.

[00183] The Growth Inhibition (GI) was utilized as a measure of cell growth. GI percentages are calculated by applying the following test and equation:

$$\text{If } T < V_0 : 100 * (1 - \frac{T - V_0}{V_0})$$
$$\text{If } T \geq V_0 : 100 * (1 - \frac{T - V_0}{V - V_0})$$

[00184] where T is the signal measure for a test article at 72 or 96 hours, V is the untreated/vehicle-treated control measure, and Vo is the untreated/vehicle control measure at time zero (also colloquially referred to as T0 plates). This formula is derived from the Growth Inhibition calculation used in the National Cancer Institute's NCI-60 high throughput screen.

[00185] A GI reading of 0% represents no growth inhibition and would occur in instances where the T reading at 72 or 96 hours are comparable to the V reading at the respective time period. A GI 100% represents complete growth inhibition (cytostasis) and in this case cells treated with compound for 72 or 96 hours would have the same endpoint reading as T0 control cells. A GI of 200% represents complete death (cytotoxicity) of all cells in the culture well and in this case the T reading at 72 or 96 hours will be lower than the T0 control (values near or at zero). These GI calculations were used in all single agent and combination data analysis for the ProNAi combination screen.

[00186] Inhibition as a measure of cell viability: Inhibition levels of 0% represent no inhibition of cell growth by treatment. Inhibition of 100% represents no doubling of cell numbers

during the treatment window. Both cytostatic and cytotoxic treatments can yield an Inhibition Percentage of 100%. Inhibition Percentage is calculated as the following: I=1-T/U, where T is treated and U is untreated.

Synergy Score Analysis

[00187] To measure combination effects in excess of Loewe additivity, a scalar measure was used to characterize the strength of synergistic interaction termed the Synergy Score. The Synergy Score is calculated as:

$$Synergy\ Score = \log f_X \log f_Y \sum \max(0, I_{data})\,(I_{data} - I_{Loewe})$$

[00188] The fractional inhibition for each component agent and combination point in the matrix is calculated relative to the median of all vehicle-treated control wells. The Synergy Score equation integrates the experimentally-observed activity volume at each point in the matrix in excess of a model surface numerically derived from the activity of the component agents using the Loewe model for additivity. Additional terms in the Synergy Score equation (above) are used to normalize for various dilution factors used for individual agents and to allow for comparison of synergy scores across an entire experiment. The inclusion of positive inhibition gating or an Idata multiplier removes noise near the zero effect level, and biases results for synergistic interactions at that occur at high activity levels. Combinations with higher maximum Growth Inhibition (GI) effects or those which are synergistic at low concentrations will have higher Synergy Scores. Those combinations with Synergy Scores that statistically supersede baseline self-cross values can be considered synergistic. The magnitude of GI effects may be linked to the growth rate of cells which varies for each cell line examined (Figure 2, Appendix 1 and Appendix 2, incorporated into and part of this specification).

[00189] Potency shifting was evaluated using an isobologram, which demonstrates how much less drug is required in combination to achieve a desired effect level, when compared to the single agent doses needed to reach that effect. The isobologram was drawn by identifying the locus of concentrations that correspond to crossing the indicated inhibition level. This is done by finding the crossing point for each single agent concentration in a dose matrix across the concentrations of the other single agent. Practically, each vertical concentration C_Y is held fixed while a bisection algorithm is used to identify the horizontal concentration C_X in combination with that vertical dose that gives the chosen effect level in the response surface $\boldsymbol{Z}(C_X,C_Y)$. These

concentrations are then connected by linear interpolation to generate the isobologram display. For synergistic interactions, the isobologram contour fall below the additivity threshold and approaches the origin, and an antagonistic interaction would lie above the additivity threshold. The error bars represent the uncertainty arising from the individual data points used to generate the isobologram. The uncertainty for each crossing point is estimated from the response errors using bisection to find the concentrations where $\boldsymbol{Z}-\sigma_Z(C_X,C_Y)$ and $\boldsymbol{Z}+\sigma_Z(C_X,C_Y)$ cross $\boldsymbol{I}_{cut}$, where σ_Z is the standard deviation of the residual error on the effect scale. The Synergy Score values for each combination are shown in Figure 5.

Loewe Volume Score Analysis

[00190] Loewe Volume Score is used to assess the overall magnitude of the combination interaction in excess of the Loewe additivity model. Loewe Volume is particularly useful when distinguishing synergistic increases in a phenotypic activity (positive Loewe Volume) versus synergistic antagonisms (negative Loewe Volume). When antagonisms are observed, as in the current dataset, the Loewe Volume is assessed to examine if there is any correlation between antagonism and a particular drug target-activity or cellular genotype. This model defines additivity as a non-synergistic combination interaction where the combination dose matrix surface is indistinguishable from either drug crossed with itself.

[00191] The calculation for additivity is: I_{Loewe} that satisfies $(X/X_I) + (Y/Y_I) = 1$ where X_I and Y_I are the single agent effective concentrations for the observed combination effect I. For example, if 50% inhibition is achieved separately by 1μM of drug A or 1μM of drug B, a combination of 0.5μM of A and 0.5μM of B should also inhibit by 50%.

[00192] Activity observed in excess of Loewe additivity identifies synergistic interaction. For the present analysis, empirically derived combination matrices were compared to their respective Loewe additivity models constructed from experimentally collected single agent dose response curves. Summation of this excess additivity across the dose response matrix is referred to as Loewe Volume. Positive Loewe Volume suggests synergy, while negative Loewe Volume suggests antagonism (Figure 3). As mentioned above, Synergy Score is a positively gated value and cannot be used to gauge potential antagonism.

[00193] Synergy can be quantified in relation to the Loewe additivity shape model (Null Interaction Model) which is constructed from the single agent dose responses. The additivity

model serves as a "null-hypothesis" and assumes no synergistic interaction between the enhancee and the enhancer. To quantify Loewe Volume, the empiric data surface is subtracted from the additivity shape model (Null Interaction Model). Loewe Volume is the summation of any residual excess activity across the combination dose matrix and can be used to measure both potential synergy (positive Loewe Volume) and antagonism (negative Loewe Volume). Synergy Score is a positively gated value, and cannot be used to gauge potential antagonism. Scores in Figure 3 were calculated using the logistic curve fit.

Example 1: Combination Drug Screening.

Single Agent Assessment

[00194] ProNAi Compound 1 single agent activity in each of the dosing schedules was collected as part of the combination screen. The single agent data can be visualized by the single agent dose response chart and single agent measures such as GI50, IC50, Maximum Response Observed (Max Response) were calculated (Figure 2 and Appendix 2 incorporated into and part of this specification). The activity of ProNAi Compound 1 in the co-treatment schedule (Appendix 2) was assessed.

[00195] Chalice Analyzer allows two ways of visualizing single agent dose response curves. The Logistics Curve fit modeling in Analyzer uses sigmoidal modeling of the data points. In most contexts, the Logistics Curve fit will accurately model the dose response curve. All subsequent analyses for the single agent and combination activities of ProNAi Compound 1 contained within this Example use the Linear Interpolation Curve fit.

Combination Screens

[00196] A drug combination screen was performed with SRA737/ProNAi Compound 1 combined with enhancer compounds in a cell line panel. Two dosing schedules for the drug combination were evaluated, co-treatment schedule and sequential schedule. Combination data was collected using a 6 x 6 optimized dose matrix format (Figure 4) across the cell line panel. The starting concentration and fold dilution of all compounds was selected for each cell line by scientists based upon reference data. The enhancer single agent dose curve is shown on the vertical axis. The enhancee single agent dose curve is shown along the horizontal axis. For each

6 x 6 dose matrix, the enhancee and enhancer are collected in a single agent dose series of 5 points (plus the zero) and a total of 13 combination dose ratio points are collected.

[00197] The Synergy Score values for each combination are summarized in Figure 5. A number of overall general trends of combination activities are observed: Combination activities with ProNAi Compound 1 are both cell line and compound specific. In particular, relatively high Synergy Scores are observed in both treatment schedules with the Wee1 inhibitor, MK 1775. In contrast, several cell lines demonstrate 'resistance' to combination activities. The observation that certain cell lines are 'more prone' to strong combination activities and demonstrate relatively high Synergy Scores, while others are resistant, suggests that the genomic background and molecular and signaling changes that are specific to these cell lines could potentiate or antagonize the synergistic activity of ProNAi Compound 1. Of note, although ProNAi Compound 1 demonstrated only minimal single agent activity in the squamous cell carcinoma CAL-27 cell line, some of the strongest synergies with this enhancee were observed in this cell line (Figure 5). Mutations in TP53 and NRAS that occur in this cell line could contribute to the genomic instability and genotoxic stress, and therefore potentiate the synergistic activity of ProNAi Compound 1.

Target Cluster Analysis

[00198] The enhancer library contains compounds that can be clustered by their target or mode-of-action. Such clustering serves as a useful method to observe similar patterns of activity across the cell line panel and, when present, may implicate a particular cell line genotype or network context that is permissive for synergistic interaction between two targets. Target or mode-of-action information was used to cluster like-annotated enhancers across the cell line panel and matrices with high-to-moderate scores reviewed to select the cut-off, using the histogram analysis function in Chalice Analyzer. This strategy allows inclusion of combination activities which might not be strong, but are potentially valid and interesting. These moderate synergies can be the result of compound behavior in specific cell lines [for example, steep dose response(s) for enhancer or enhancee or reduced compound activities linked to growth rate]. Moreover, only weak or moderate synergies might occur as a result of genetic or epigenetic differences that affect signaling pathway activation status and thereby compound activity. The strategy of using target or pathway cluster profiles with visual inspection of matrices lends

support to subtle synergies that might be otherwise overlooked and provides additional detail about the breadth-of-activity of a particular target across the cell line panel.

[00199] The matrix view in Chalice Analyzer provides a framework for studying data trends; however, definitive conclusions were made only after careful examination of individual matrices. With consideration of these caveats, the matrix view was used to quickly identify and assess combination effects for the cell line panel. The focus on specific mechanistic classes can be quite powerful, as there are consistent trending which supports the observed effects.

[00200] The following sections focus upon target cluster analysis of the combination screen. The matrix views for the 15 cell lines included in the combination screen using the Growth Inhibition measure and linear interpolation curve fit are provided. The matrix views shown also indicate the data collected from both drug treatment schedules: co-treatment (72 hours treatment time) and sequential (96 hours treatment time).

DNA damage response

[00201] Diverse responses are elicited in cells following DNA damage. Depending on the extent of the damage, a signaling cascade is activated which causes cell cycle arrest and initiates repair of damage. Importantly, inhibiting the repair of DNA could potentiate the antitumor efficacies of various DNA damaging chemotherapeutic agents. Inhibitors of the DNA damage response are included in this study: the Wee1 inhibitor, MK 1775 and the DNA-PK inhibitor, NU 7441.

[00202] Strong-to-moderate synergies were observed across the cell line panel and in both treatment schedules when ProNAi Compound 1 was combined with MK 1775 (Figure 5, Figure 6). Figure 5 shows Synergy scores for each cell line tested. Synergy Score cut-off of 3.0 was made for the analysis of the co-treatment schedule with Synergy Scores from 3.0-6.0 highlighted in lighter shades of grey while scores above 6.0 are highlighted in dark grey. A Synergy Score cut-off of 3.0 was made for the analysis of the sequential treatment schedule with Synergy Scores from 3.0-4.5 highlighted in a lighter shade of grey while scores above 4.5 are highlighted in dark grey. The Growth Inhibition dose matrices with the two highest Synergy Scores in both dosing schedules are shown in Figure 6. These results confirm the synergistic activity of Chk1 inhibition by SRA737 and Wee1 inhibition for many cancer indications.

Appendix 1. Cell Line Panel

Cell Line	Indication	Cell Density	Doubling Time Median	Growth Medium
5637	Bladder	500	24.1	RPMI with 10% FBS
J82		500	34.1	EMEM with 10% FBS
TCCSUP		500	19.6	MEM in Earle's BSS with 10% FBS, Non-Essential Amino Acids and 1mM sodium pyruvate
HT-29	Colorectal	500	23.1	McCoy's 5A and 10% FBS
SW620		500	19.6	RPMI with 10% FBS (5% CO2)
CAL-27	Head and Neck	500	37.9	DMEM with 10% FBS
FaDu		1500	26.8	Eagle's MEM with 10% FBS
Calu-6	Lung	500	29.4	Eagle's MEM with 10% FBS
NCI-H520		500	29.4	RPMI with 10% FBS
KURAMOCHI	Ovary	1500	57.1	RPMI with 10% FBS
OVCAR-3		500	53.9	RPMI with 20% FBS and 0.01 mg/ml bovine insulin
OV90		500	30.4	MCDM 105 and Medium 199 (1:1) with 15% FBS
MIA PaCa-2	Pancreas	500	25.2	DMEM with 10% FBS and 2.5% horse serum
Panc 03.27		500	31.2	RPMI with 15% FBS and 10 units/ml human recombinant insulin
SNU-324		500	52.0	RPMI with 10% FBS and 25mM HEPES and 25mM sodium bicarbonate

Appendix 2. Single Agent Dose Response Analysis Across the Cell Line Panel

EC_{50} AND GI_{50} VALUES AS MEASURED USING THE LINEAR INTERPOLATION FIT CURVE

	Indication	Cell Line	EC_{50} (uM)	GI_{50} (uM)
ProNAi Compound 1	Colorectal	SW620	4.09	8.49
		HT-29	0.64	0.71
	Lung	Calu-6	0.35	0.35
		NCI-H520	3.39	6.98
	Ovary	OV90	0.41	0.60
		KURAMOCHI	0.82	1.47
		OVCAR-3	2.02	2.47
	Bladder	5637	1.49	1.63
		TCCSUP	0.22	0.23
		J82	0.35	0.93
	Pancreas	MIA PaCa-2	0.55	2.01
		SNU-324	0.02	4.37
		Panc 03.27	0.46	0.25
	Head and neck	CAL-27	0.00	
		FaDu	0.01	2.24

Example 2: Combination Assessment of Chk1 inhibitors.

[00203] Synergistic activity of structurally distinct Chk1 inhibitors with PARP inhibitors is confirmed by assessing the single agent activity of individual Chk1 inhibitors in 15 cancer cell lines (SW620, HT-29, Calu-6, NCI-H520, OV90, KURAMOCHI, OVCAR-3, 5637, TCCSUP, J82, MIA PaCa-2, SNU-324, Panc 03.27, CAL-27 and FaDu) as described above, followed by assessing combination activity of each Chk1 inhibitor with at least one Wee1 inhibitor selected from the group consisting of: MK 1775, PD166285, PF00120130, 4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3-(2H,6H)-dione (PHCD), ADC-730, ADC-999, and PD0407824. Synergy score analysis and Loewe volume score analysis is performed as described above. Structurally distinct Chk1 inhibitors include, but are not limited to: SRA737, Prexasertib (LY2606368), PF-477736, AZD7762, Rabusertib (LY2603618), MK-8776 (SCH 900776), CHIR-124, SAR-020106 or CCT245737.

Example 3: Combination Methods of Treating Tumor Growth in Humans

[00204] A human subject with a tumor is treated with a combination of a Chk1 inhibitor and a Wee1 inhibitor resulting in a reduction of tumor growth.

[00205] A subject in need of treatment is selected or identified. The identification of the subject can occur in a clinical setting. The subject has a tumor resulting from a cancer, *e.g.*, the subject has bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, mesothelioma, melanoma, myeloma, ovarian cancer, prostate cancer, pancreatic cancer, renal cell cancer, small cell lung cancer, or squamous cell carcinoma of the head and neck.

[00206] At time zero, a suitable first dose of each of a Chk1 inhibitor and a Wee1 inhibitor is administered to the subject, either separately or in combination. The Wee1 inhibitor is, *e.g.*, MK 1775, PD166285, PF00120130, 4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3-(2H,6H)-dione (PHCD), ADC-730, ADC-999, or PD0407824. The Chk1 inhibitor is, *e.g.*, SRA737, Prexasertib (LY2606368), PF-477736, AZD7762, Rabusertib (LY2603618), MK-8776 (SCH 900776), CHIR-124, SAR-020106 or CCT245737. The SRA737 and Wee1 inhibitor are formulated as described herein.

[00207] After a period of time following the first dose, the subject's condition is evaluated, *e.g.*, by measuring tumor growth. This measurement can be accompanied by a measurement of expression of a marker gene in a cell, of inhibiting a Chk1 activity in a cell, and of activating a CDC25 and CDK1/2 activity in a cell. Other relevant clinical endpoints are also measured as described herein.

[00208] The number and strength of doses are adjusted according to the subject's needs.

[00209] After treatment, the subject's tumor growth rate is lowered relative to the rate existing prior to the treatment, or relative to the rate measured in a similarly afflicted but untreated subject.

[00210] While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it are understood by persons skilled in

the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

[00211] All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

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CLAIMS

1. A method of inhibiting the growth of a tumor in a subject in need thereof, comprising administering to the subject a first effective amount of a SRA737 and a second effective amount of a Wee1 inhibitor.

2. The method of claim 1, wherein the SRA737 and the Wee1 inhibitor are administered separately.

3. The method of claim 2, wherein the Wee1 inhibitor is administered at least twenty-four (24) hours after the administration of SRA737.

4. The method of any one of claims 1 through 3, wherein the SRA737 and the Wee1 inhibitor are administered; and subsequently both SRA737 and the Wee1 inhibitor are administered continuously for at least twenty-four (24) hours.

5. The method of any one of claims 1 through 3, wherein the SRA737 and the Wee1 inhibitor are administered; and subsequently either one of, or both of, SRA737 and/or the Wee1 inhibitor is separately administered intermittently for at least twenty-four (24) hours.

6. The method of any one of claims 1 through 5, wherein the Wee1 inhibitor is selected from the group consisting of: MK 1775, PD166285, PF00120130, 4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3-(2H,6H)-dione (PHCD), ADC-730, ADC-999, and PD0407824.

7. The method of any one of claims 1 through 5, wherein the Wee1 inhibitor is MK 1775.

8. The method of any one of claims 1 through 7, wherein the tumor is in a subject suffering from a condition or disorder selected from the group consisting of: bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, mesothelioma, melanoma, myeloma, ovarian cancer, prostate cancer, pancreatic cancer, renal cell cancer, small cell lung cancer, and squamous cell carcinoma of the head and neck.

9. The method of any one of claims 1 through 8, wherein the subject has a mutation in at least one gene selected from the group consisting of: a tumor suppressor gene, a DNA damage repair gene, a replication stress gene, or an oncogenic driver gene.

10. The method of any one of claims 1 through 9, wherein the route of administration is selected from the group consisting of: intravenous, subcutaneous, cutaneous, oral, intramuscular, and intraperitoneal.

11. The method of any one of claims 1 through 10, wherein the first effective amount is 0.001 mg/kg to 15 mg/kg and the second effective amount is 0.001 mg/kg to 15 mg/kg.

12. The method of claim 11, wherein the first effective amount is 0.1 mg/kg to 1.5 mg/kg and the second effective amount is 0.1 mg/kg to 1.5 mg/kg.

13. The method of claim 11, wherein the first effective amount is 10 mg to 1000 mg.

14. The method of any one of claims 1 through 13, wherein tumor growth is reduced in the subject.

15. The method of any one of claims 1 through 14, wherein tumor growth is reduced by at least 1% after administration.

16. The method of any one of claims 1 through 15, wherein administration results in tumor growth of no more than 5% of the original tumor volume after administration.

17. The method of any one of claims 1 through 16, wherein the subject is human.

18. A method of inhibiting a Chk1 activity in a cell, the method comprising contacting the cell with a first effective amount of SRA737 and a second effective amount of a Wee1 inhibitor.

19. A method of activating CDC25 activity in a cell, the method comprising contacting the cell with a first effective amount of SRA737 and a second effective amount of a Wee1 inhibitor.

20. A method of activating CDK1/2 activity in a cell, the method comprising contacting the cell with a first effective amount of SRA737 and a second effective amount of a Wee1 inhibitor.

21. The method of any one of claims 18 through 20, wherein the cell is a tumor cell.

22. The method of any one of claims 18 through 21, wherein the method is performed *in vitro*.

23. The method of any one of claims 18 through 22, wherein the SRA737 and the Wee1 inhibitor are administered simultaneously.

24. The method of any one of claims 18 through 23, wherein the SRA737 and the Wee1 inhibitor are administered sequentially.

25. The method of any one of claims18 through 24, wherein the SRA737 and the Wee1 inhibitor are administered; and subsequently both SRA737 and the Wee1 inhibitor are administered continuously for at least twenty-four (24) hours.

26. The method of any one of claims 18 through 25, wherein the SRA737 and the Wee1 inhibitor are administered; and subsequently either one of, or both of: SRA737 or the Wee1 inhibitor is separately administered intermittently for at least twenty-four (24) hours.

27. A combination comprising SRA737 and a Wee1 inhibitor.

28. The combination of claim 27, wherein the Wee1 inhibitor is selected from the group consisting of: MK 1775, PD166285, PF00120130, 4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3-(2H,6H)-dione (PHCD), ADC-730, ADC-999, and PD0407824.

29. A pharmaceutical composition comprising the combination of claim 27 or claim 28 and at least one pharmaceutically acceptable carrier or excipient.

30. A SRA737 for use in inhibiting a tumor growth in a subject in need thereof by co-administration with a Wee1 inhibitor.

31. A Wee1 inhibitor for use in inhibiting a tumor growth in a subject in need thereof by co-administration with SRA737.

32. A product comprising SRA737 and a Wee1 inhibitor for simultaneous, separate or sequential use in the inhibition of a tumor growth in a subject in need thereof.

33. A kit comprising the combination of claim 27 or claim 28 or the pharmaceutical composition of claim 29 and instructions for use.

34. A method of inhibiting a tumor growth in a subject in need thereof, comprising administering to the subject a first effective amount of a Chk1 inhibitor and a second effective amount of Wee1 inhibitor.

35. The method of claim 31, wherein the Wee1 inhibitor is selected from the group consisting of: MK 1775, PD166285, PF00120130, 4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3-(2H,6H)-dione (PHCD), ADC-730, ADC-999, and PD0407824.

36. The method of claim 31 or 32, wherein the subject has a condition or disorder selected from the group consisting of: bladder cancer, breast cancer, colorectal cancer, esophageal cancer,

gastric cancer, head and neck cancer, hepatocellular cancer, leukemia, lung cancer, lymphoma, mesothelioma, melanoma, myeloma, ovarian cancer, prostate cancer, pancreatic cancer, renal cell cancer, small cell lung cancer, and squamous cell carcinoma of the head and neck.

37. A Chk1 inhibitor for use in inhibiting a tumor growth in a subject in need thereof by co-administration with a Wee1.

38. A Wee1 inhibitor for use in inhibiting a tumor growth in a subject in need thereof by co-administration with a Chk1 inhibitor.

39. A product comprising a Chk1 inhibitor and a Wee1 inhibitor for simultaneous, separate or sequential use in the inhibition of a tumor growth in a subject in need thereof.

40. Use of a Chk1 inhibitor in the manufacture of a medicament for treatment tumor growth in a subject in need thereof by co-administration of a Wee1 inhibitor.

41. Use of a Wee1 inhibitor in the manufacture of a medicament for treatment tumor growth in a subject in need thereof by co-administration of a Chk1 inhibitor.

42. The method of any one of claims 31-41, wherein the Chk1 inhibitor is selected from the group consisting of Prexasertib (LY2606368), PF-477736, AZD7762, Rabusertib (LY2603618), MK-8776 (SCH 900776), CHIR-124, SAR-020106 and CCT245737.

43. The method of claim 42, wherein the Chk1 inhibitor is Prexasertib (LY2606368).

44. The method of claim 42, wherein the Chk1 inhibitor is PF-477736.

45. The method of claim 42, wherein the Chk1 inhibitor is AZD7762.

46. The method of claim 42, wherein the Chk1 inhibitor is Rabusertib (LY2603618).

47. The method of claim 42, wherein the Chk1 inhibitor is MK-8776 (SCH 900776).

48. The method of claim 42, wherein the Chk1 inhibitor is CHIR-124.

49. The method of claim 42, wherein the Chk1 inhibitor is SAR-020106.

50. The method of claim 42, wherein the Chk1 inhibitor is CCT245737.

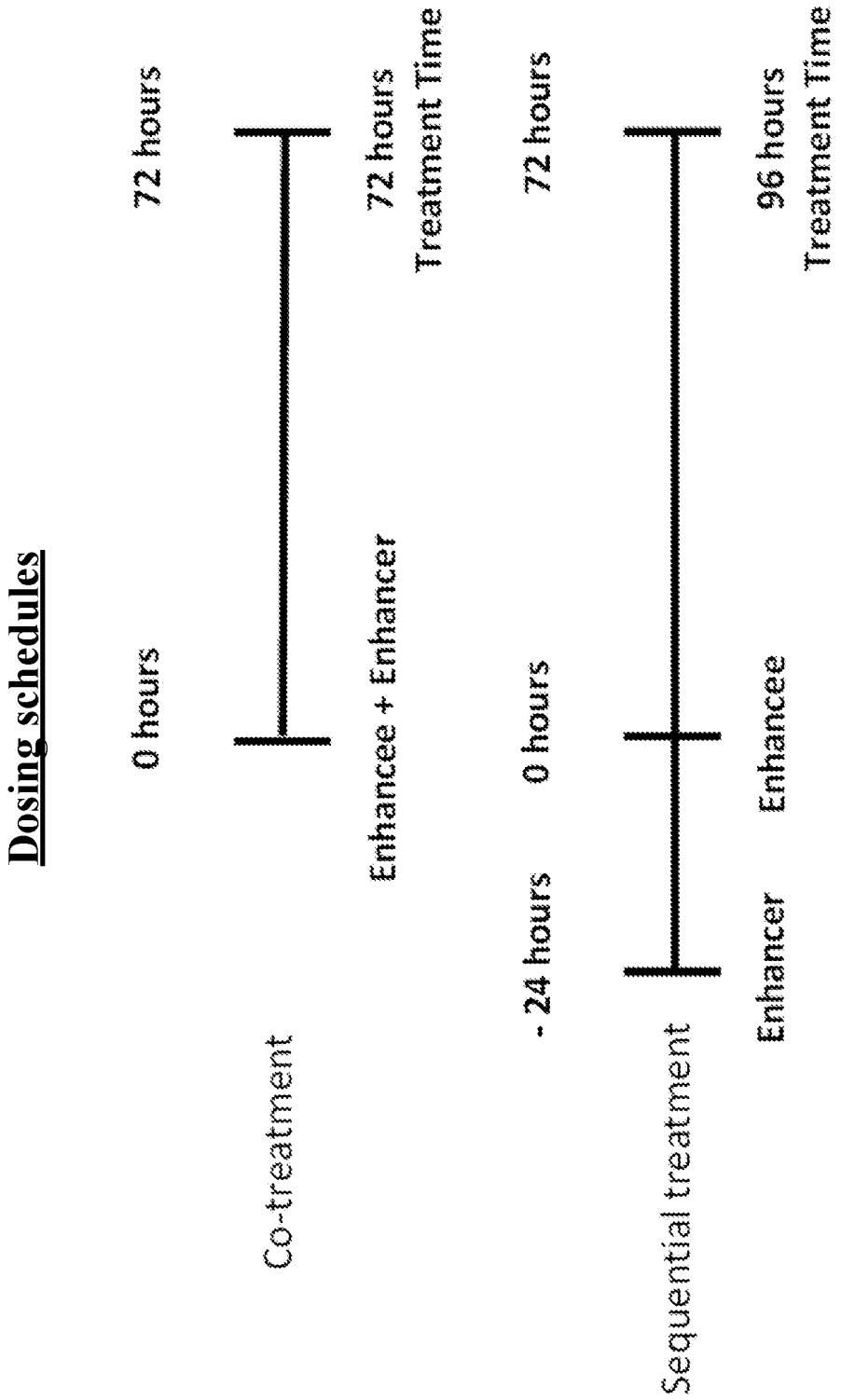

Dosing schedules
0 hours
72 hours
Co-treatment
Enhancee + Enhancer
72 hours
Treatment Time
- 24 hours
0 hours
72 hours
Sequential treatment
Enhancer
Enhancee
96 hours
Treatment Time

FIG 1

FIG. 2

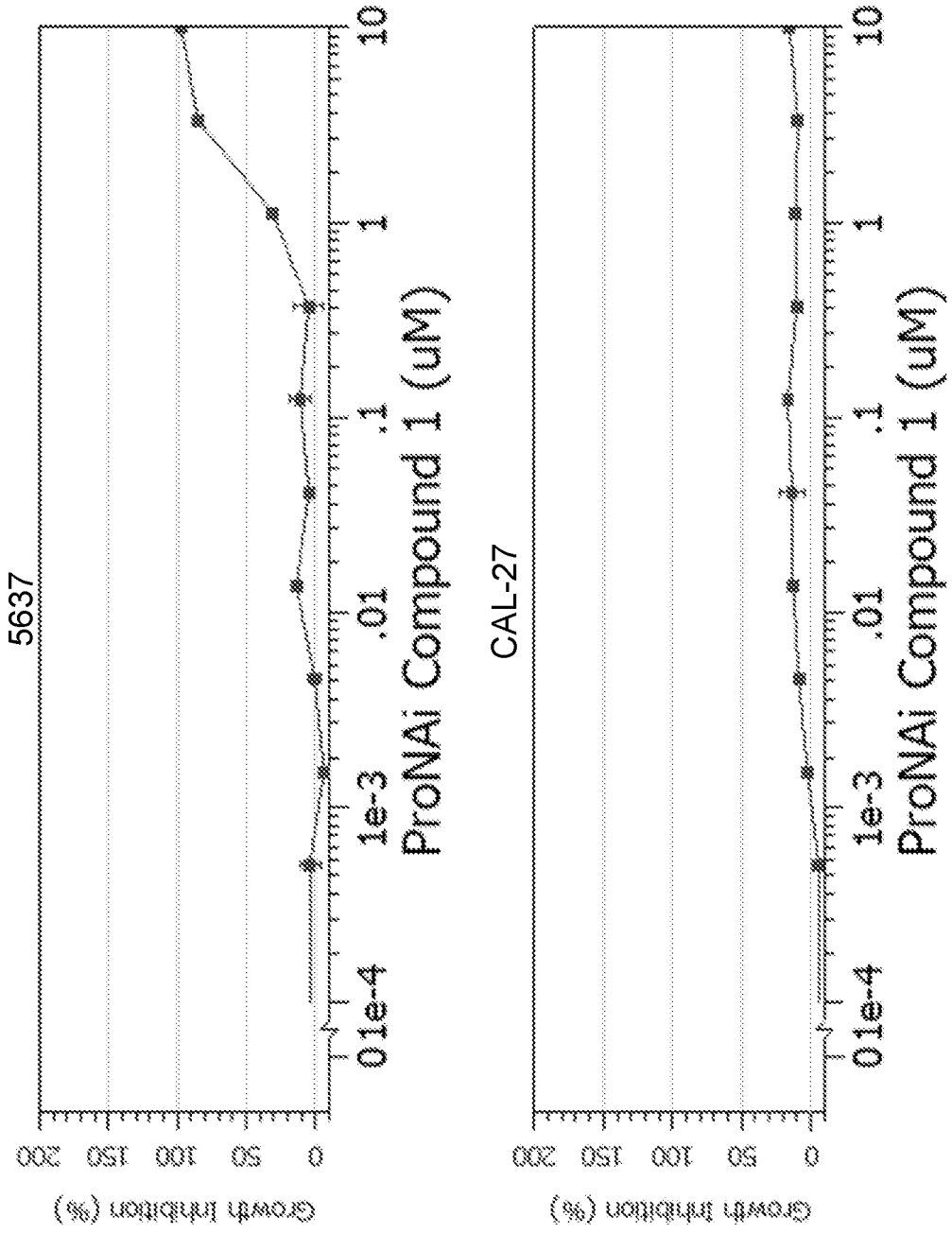
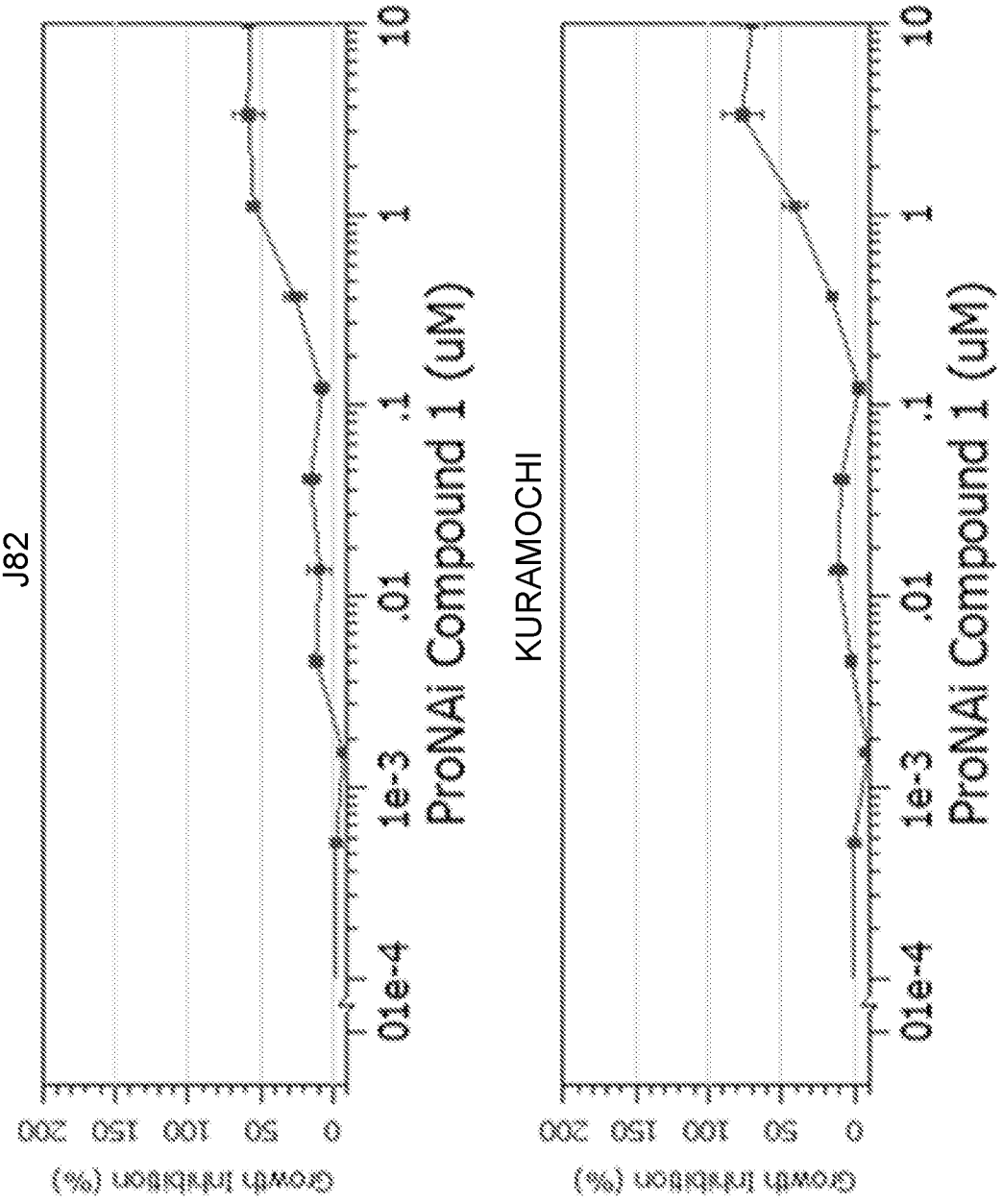

5637
CAL-27
Growth Inhibition (%)
0
50
100
150
200
01e-4
1e-3
.01
.1
1
10
ProNAi Compound 1 (uM)

FIG 2 (cont'd)


J82
KURAMOCHI
Growth Inhibition (%)
0
50
100
150
200
01e-4
1e-3
.01
.1
1
10
ProNAi Compound 1 (uM)

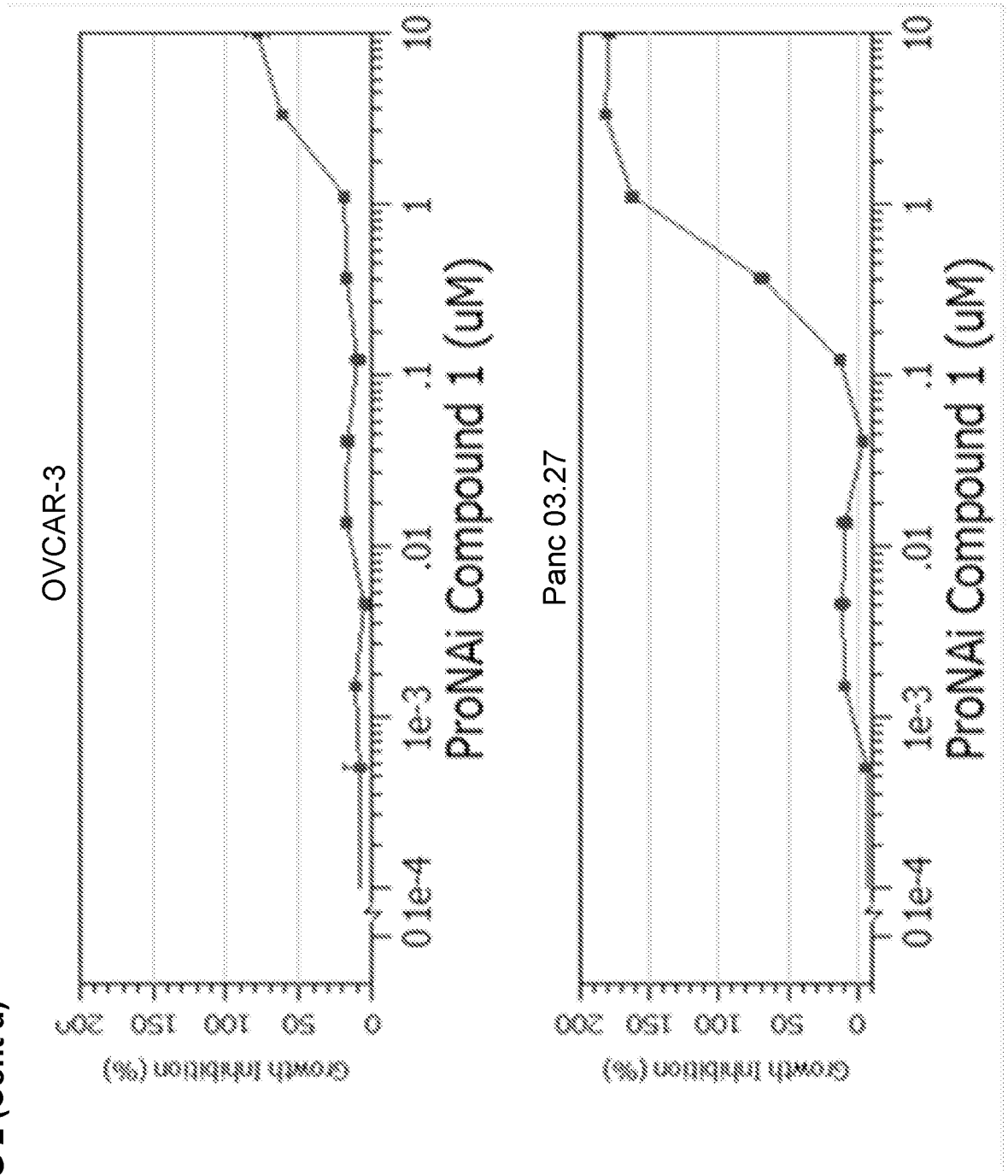

OVCAR-3
Growth Inhibition (%)
0
50
100
150
200
0
1e-4
1e-3
.01
.1
1
10
ProNAi Compound 1 (uM)
Panc 03.27
Growth Inhibition (%)
0
50
100
150
200
0
1e-4
1e-3
.01
.1
1
10
ProNAi Compound 1 (uM)

FIG 2 (Cont'd)

FIG 2 (Cont'd)

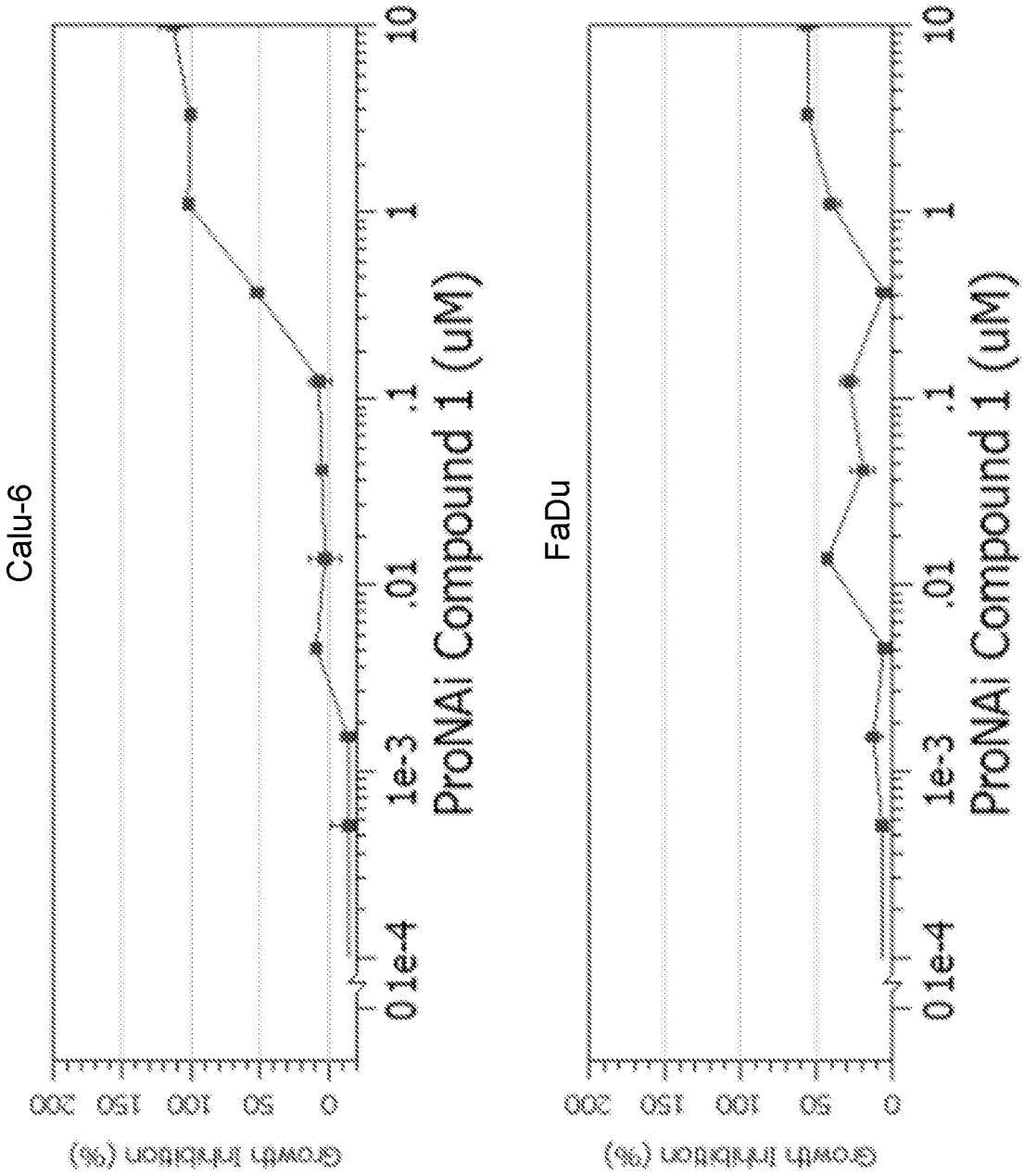

Calu-6
FaDu
Growth Inhibition (%)
0
50
100
150
200
ProNAi Compound 1 (uM)
0
1e-4
1e-3
.01
.1
1
10

FIG 2 (Cont'd)

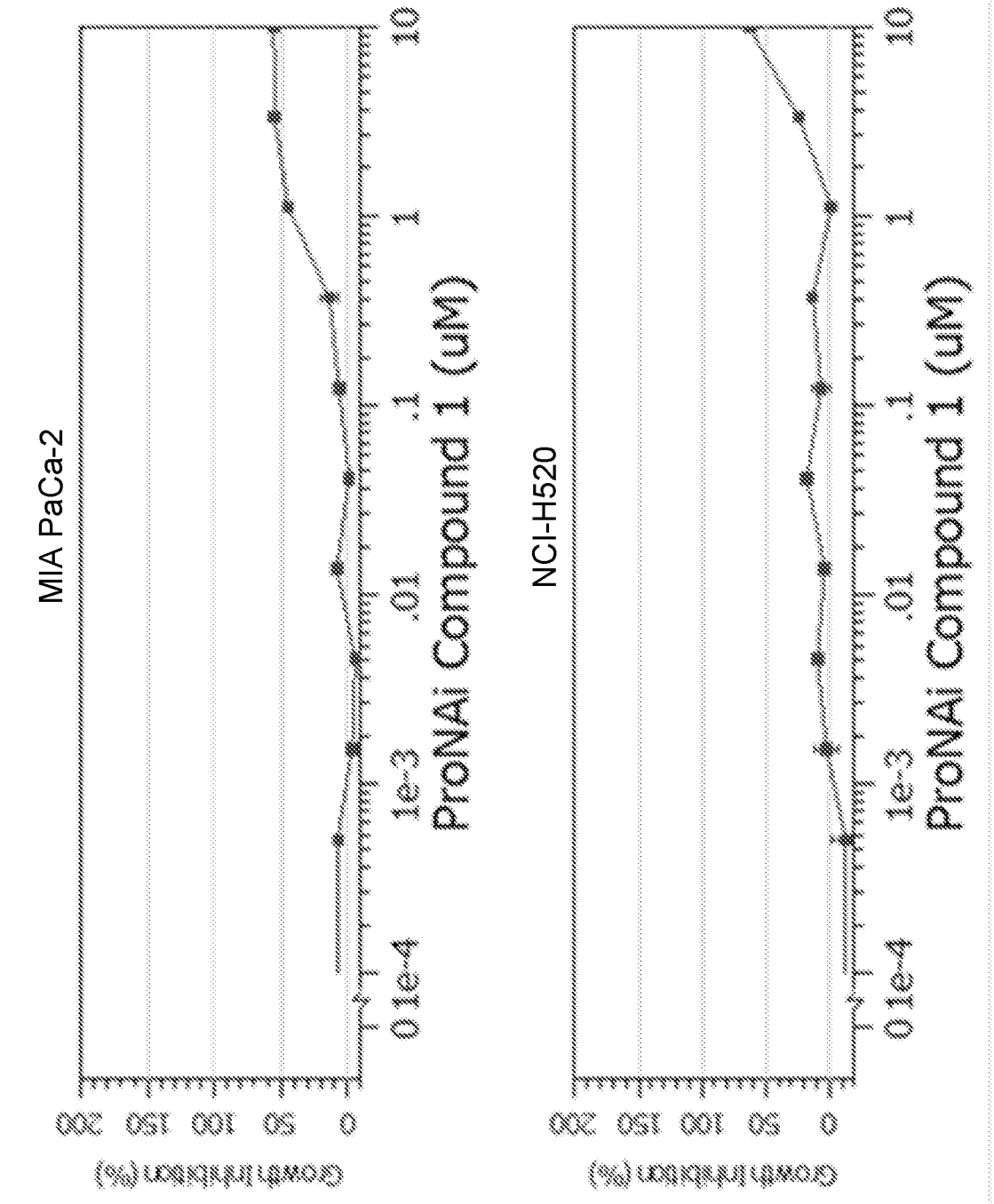

MIA PaCa-2
NCI-H520
Growth Inhibition (%)
0
50
100
150
200
ProNAi Compound 1 (uM)
0
1e-4
1e-3
.01
.1
1
10

FIG 2 (Cont'd)

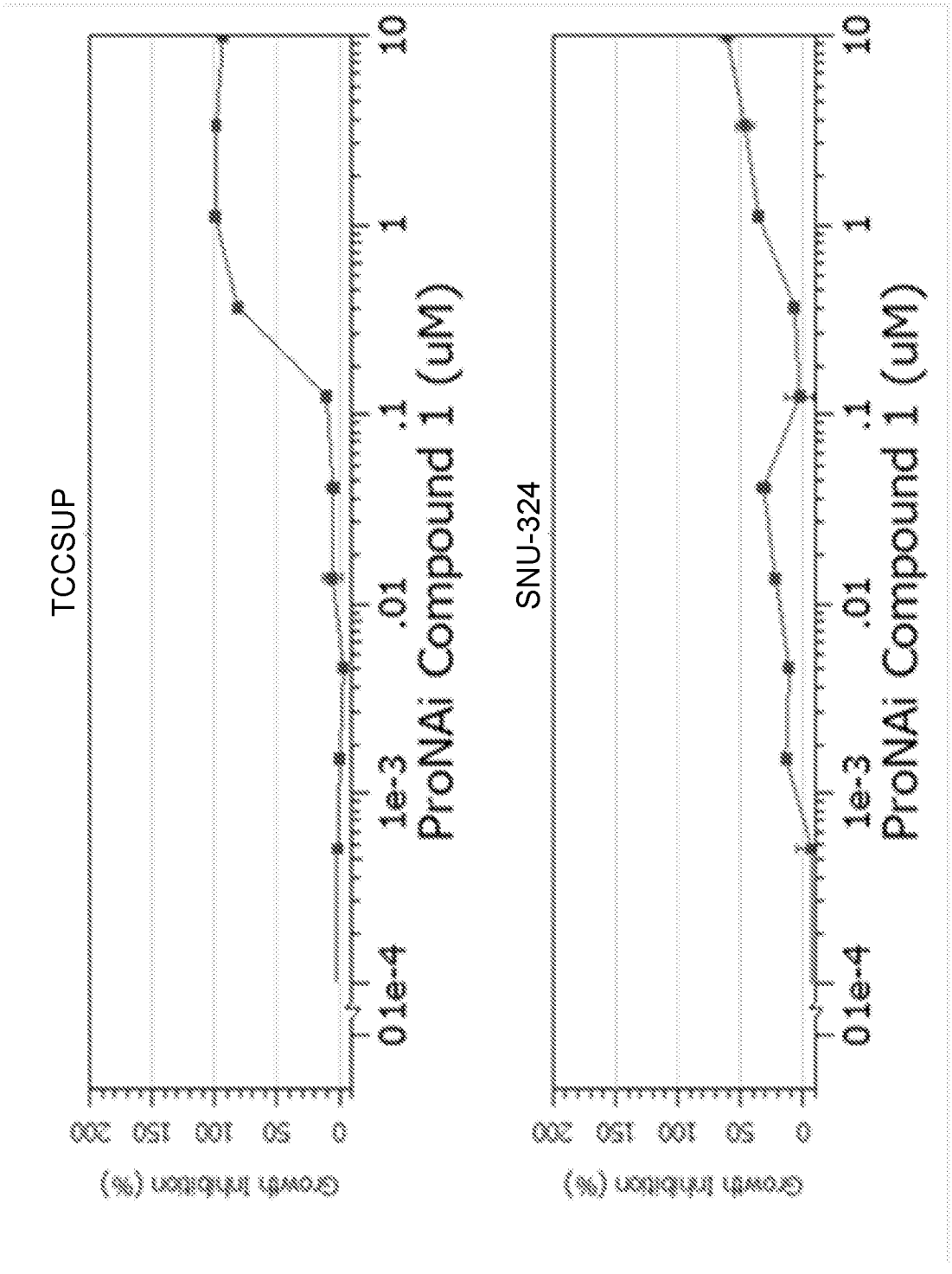
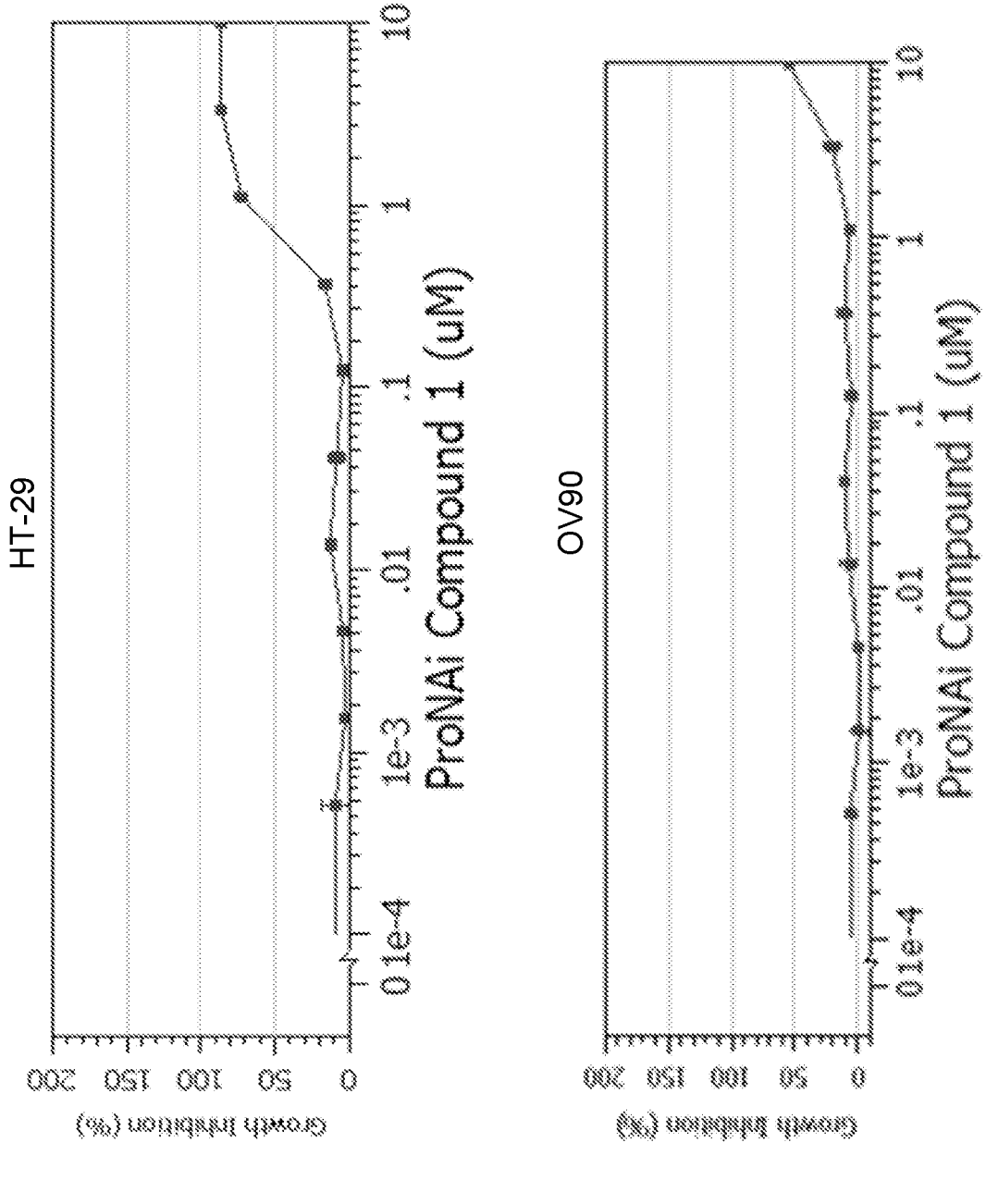

TCCSUP
SNU-324
Growth Inhibition (%)
0
50
100
150
200
01e-4
1e-3
.01
.1
1
10
ProNAi Compound 1 (uM)

FIG 2 (Cont'd)


HT-29
Growth Inhibition (%)
0
50
100
150
200
0
1e-4
.01
1e-3
.1
1
10
ProNAi Compound 1 (uM)
OV90
Growth Inhibition (%)
0
50
100
150
200
0
1e-4
.01
1e-3
.1
1
10
ProNAi Compound 1 (uM)

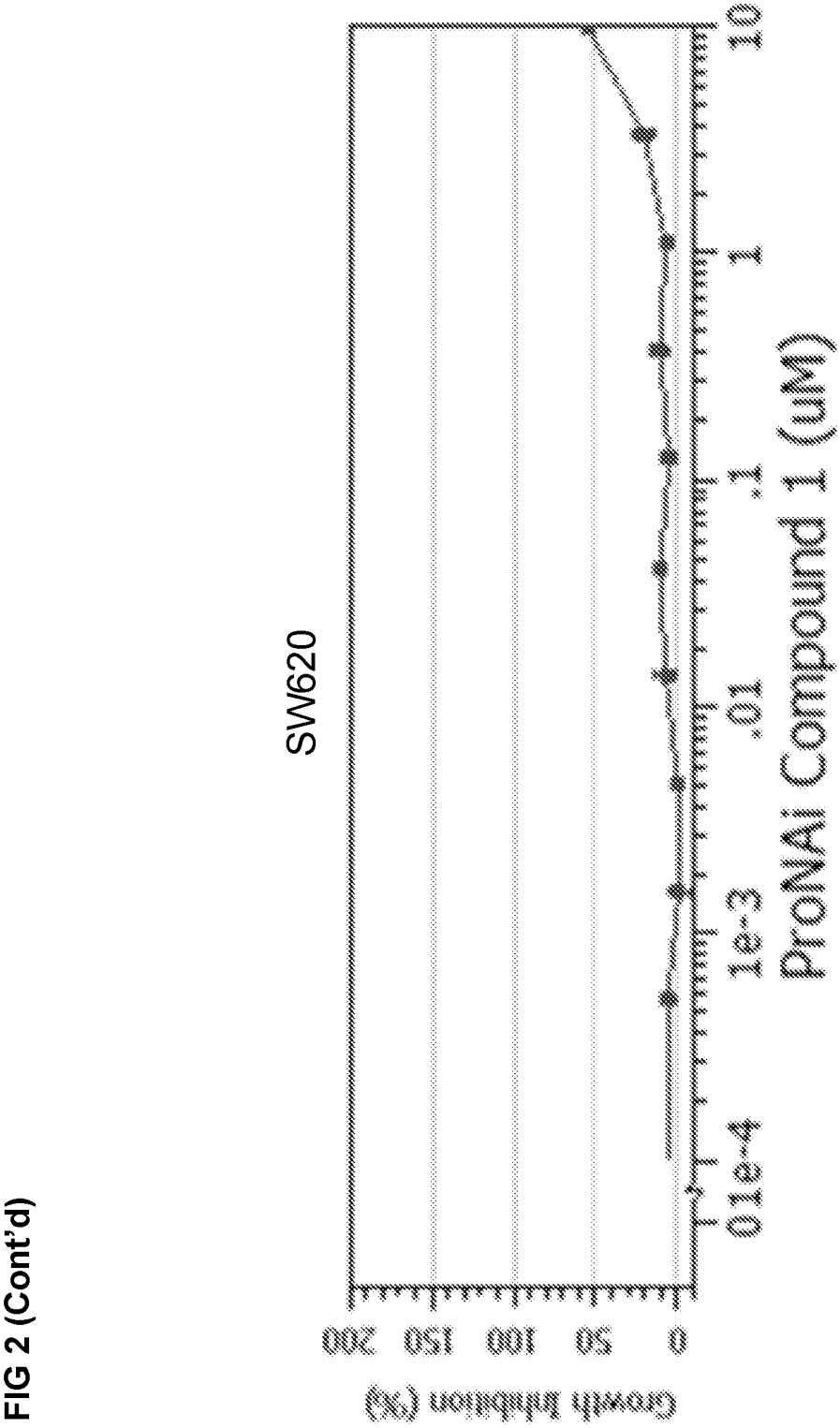

SW620
Growth Inhibition (%)
0
50
100
150
200
01e-4
1e-3
.01
.1
1
10
ProNAi Compound 1 (uM)

FIG 2 (Cont'd)

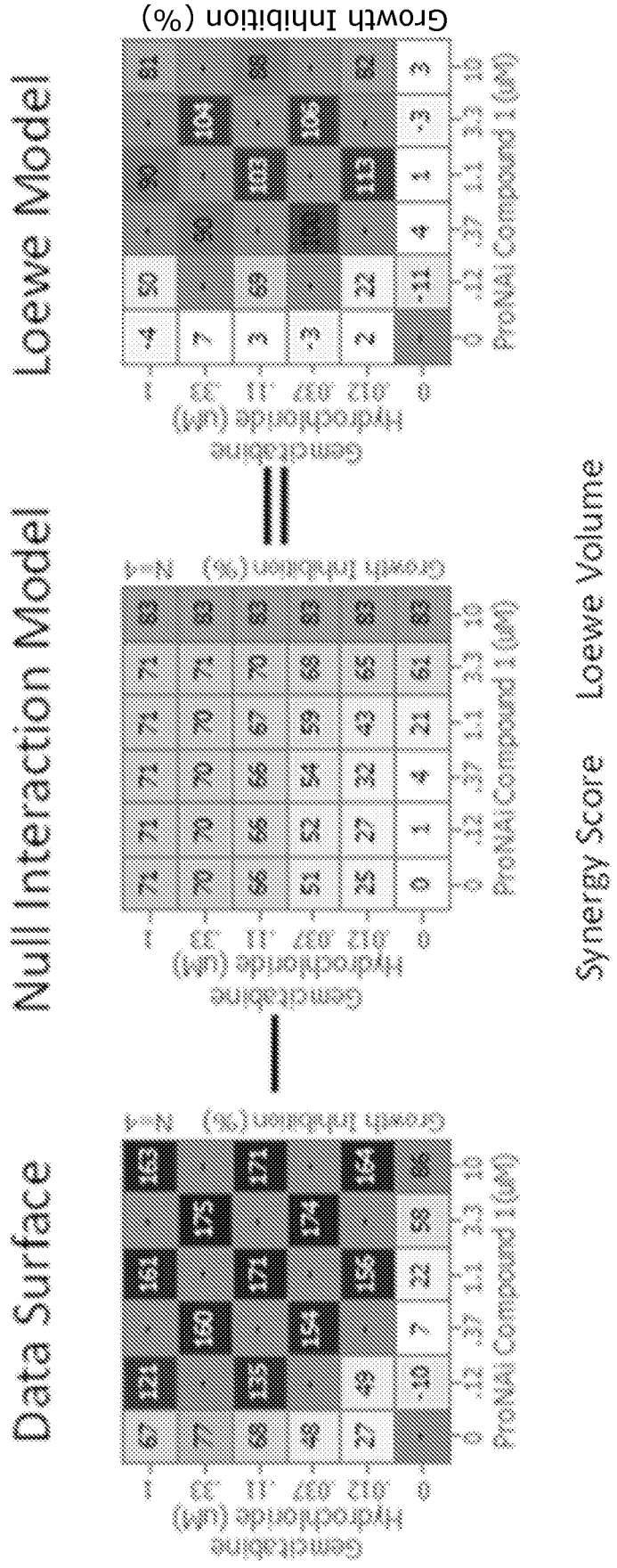

Data Surface
Null Interaction Model
Loewe Model
Growth Inhibition (%)
Gemcitabine
Hydroxytrole (uM)
ProWAI Compound 1 (uM)
Synergy Score
Loewe Volume
40.4
11

FIG 3

The 6 x 6 optimized dose matrix format

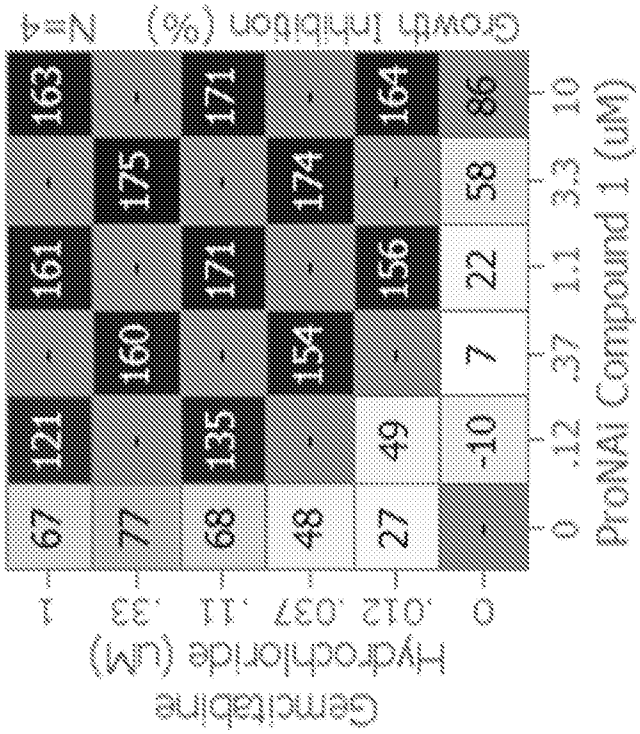
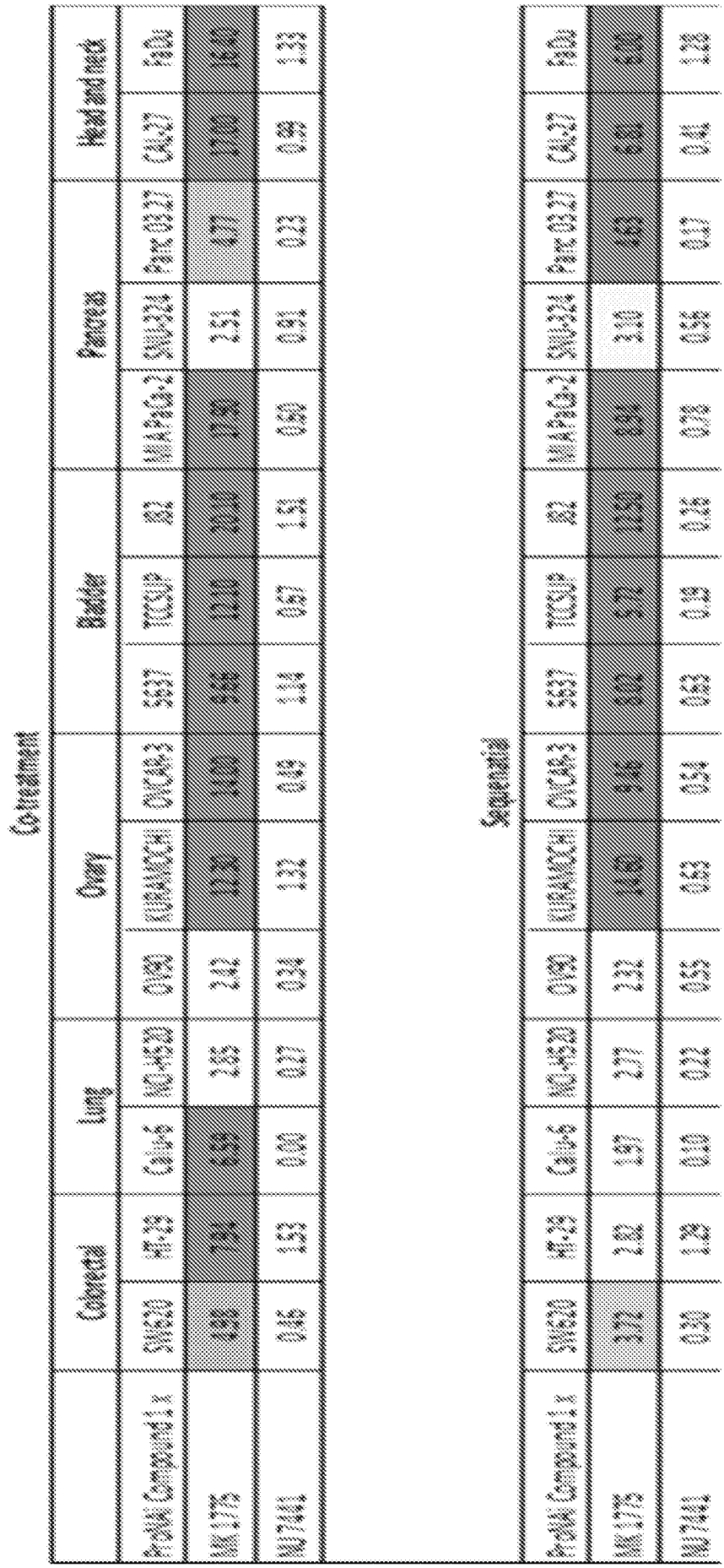


FIG 4



ProMM Compound Ix	Colorectal		Lung		Ovary		Bladder		Pancreas		Head and neck	
	SW620	HT-29	Calu-6	NCI-H520	OVCAR-3	SKOV-3	TCCSUP	J82	MIA PaCa-2	SNU-324	Panc 03.27	CAL-27	FaDu
MM-1775	1.86	1.84	2.56	1.85	2.42	4.24	4.58	4.13	4.43	2.51	4.77	4.65	4.46
MM-7441	0.46	1.53	0.00	0.17	0.34	1.32	0.49	1.14	0.67	1.31	0.60	0.91	0.23	0.29	1.33

ProMM Compound Ix	Colorectal		Lung		Ovary		Bladder		Pancreas		Head and neck	
	SW620	HT-29	Calu-6	NCI-H520	OVCAR-3	SKOV-3	TCCSUP	J82	MIA PaCa-2	SNU-324	Panc 03.27	CAL-27	FaDu
MM-1775	3.72	2.12	1.97	2.77	2.32	3.69	3.76	3.55	3.84	3.10	4.69	3.80	3.55
MM-7441	0.30	1.29	0.10	0.22	0.55	0.63	0.54	0.63	0.13	0.26	0.78	0.56	0.17	0.44	1.28

FIG 5

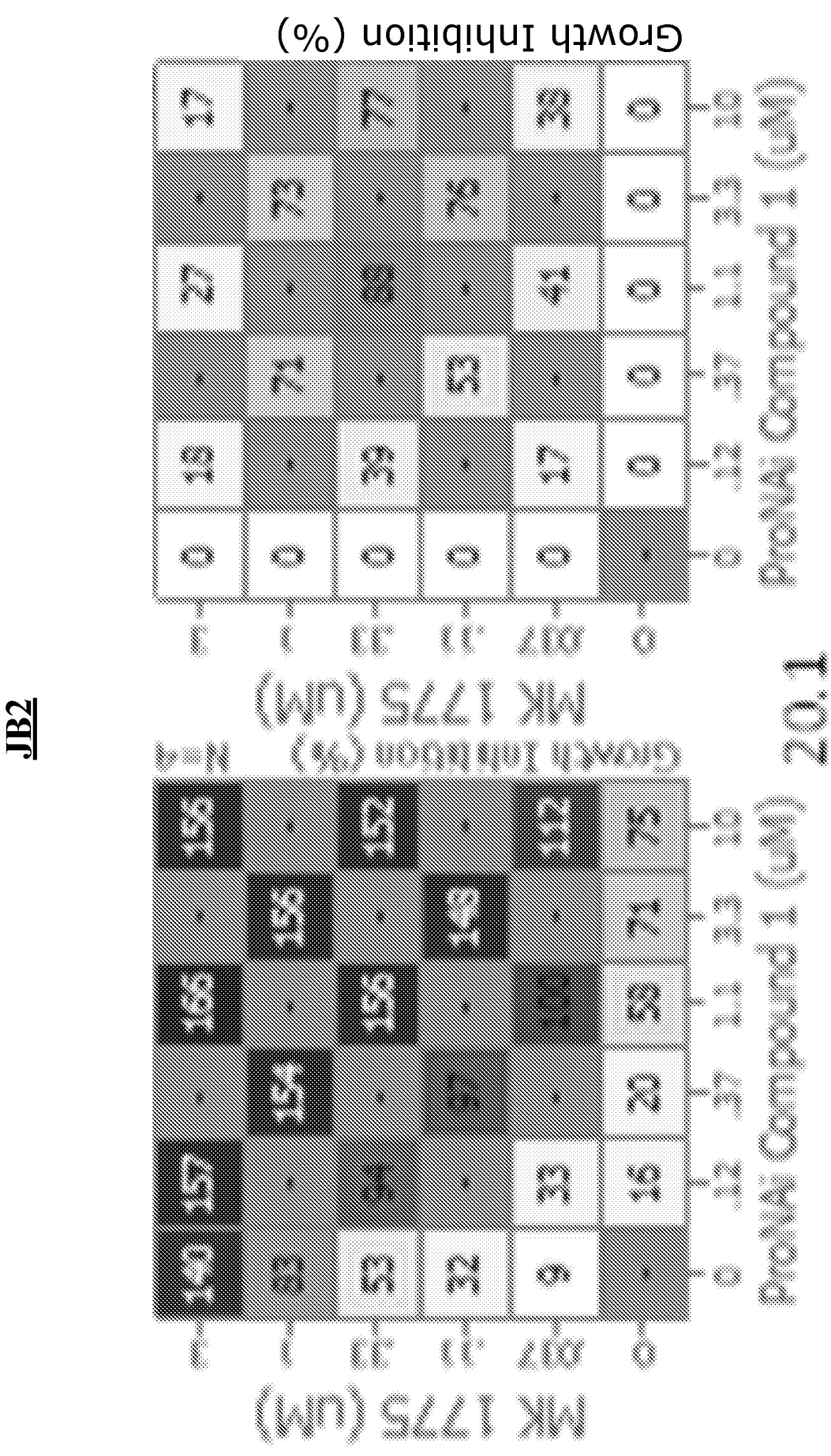

JB2
Growth Inhibition (%)
MK 1775 (uM)
ProWAi Compound 1 (uM)
Growth Inhibition (%)
MK 1775 (uM)
ProWAi Compound 1 (uM)

FIG 6

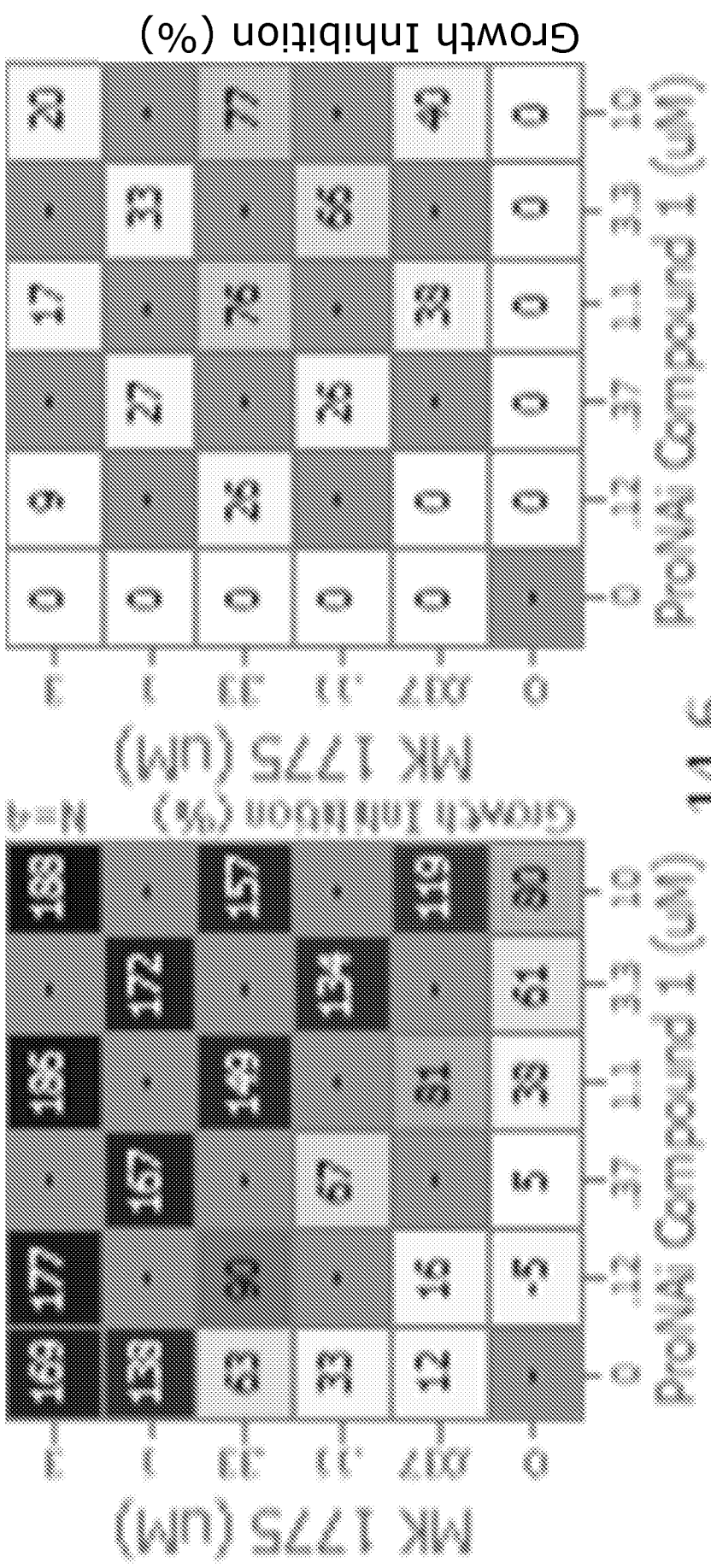


FIG 6 (Cont'd)

14.6

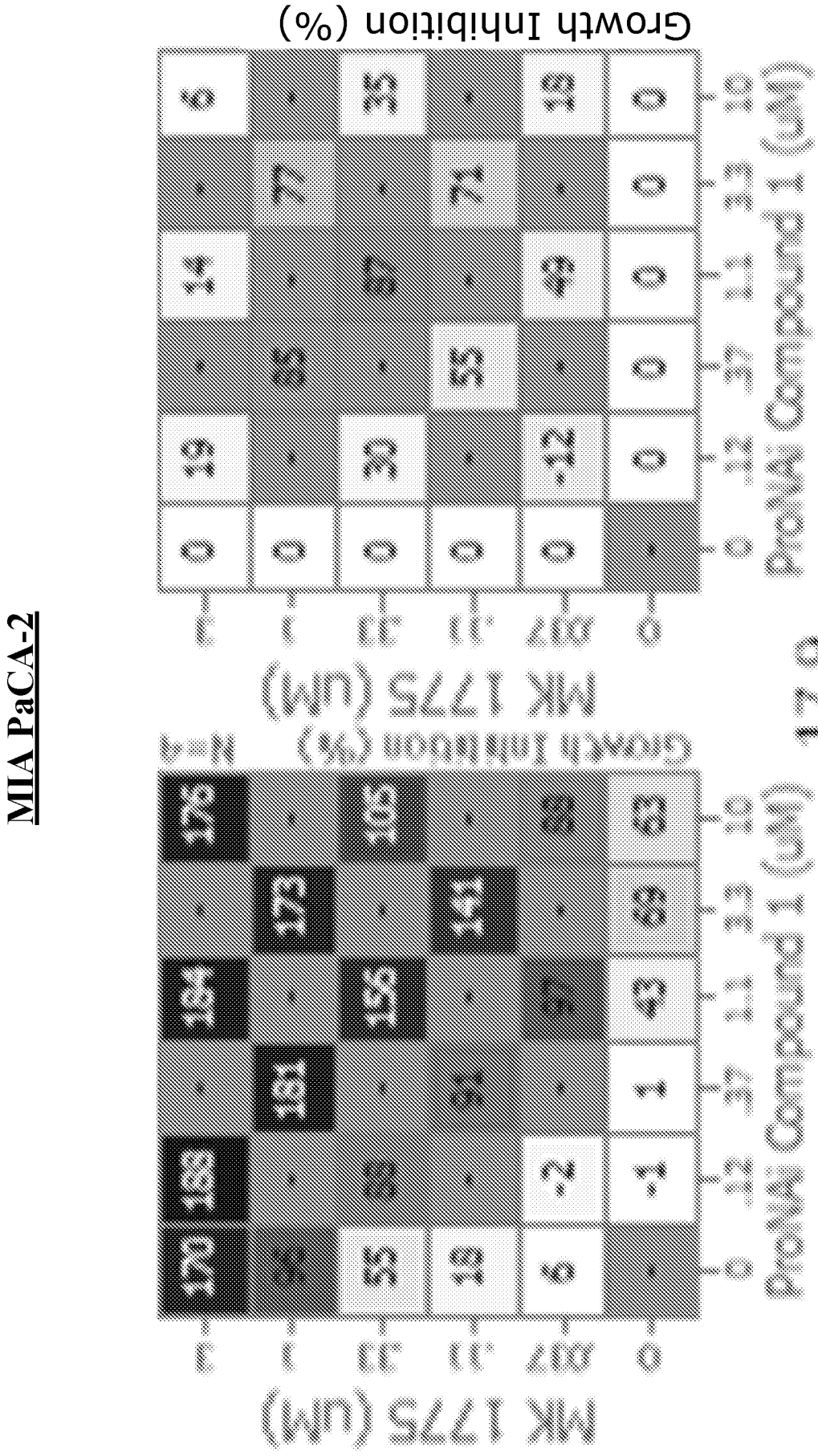

MIA PaCA-2
Growth Inhibition (%)
MK 1775 (uM)
ProWi Compound 1 (uM)
Growth Inhibition (%)
MK 1775 (uM)
ProWi Compound 1 (uM)

FIG 6 (Cont'd)

JB2

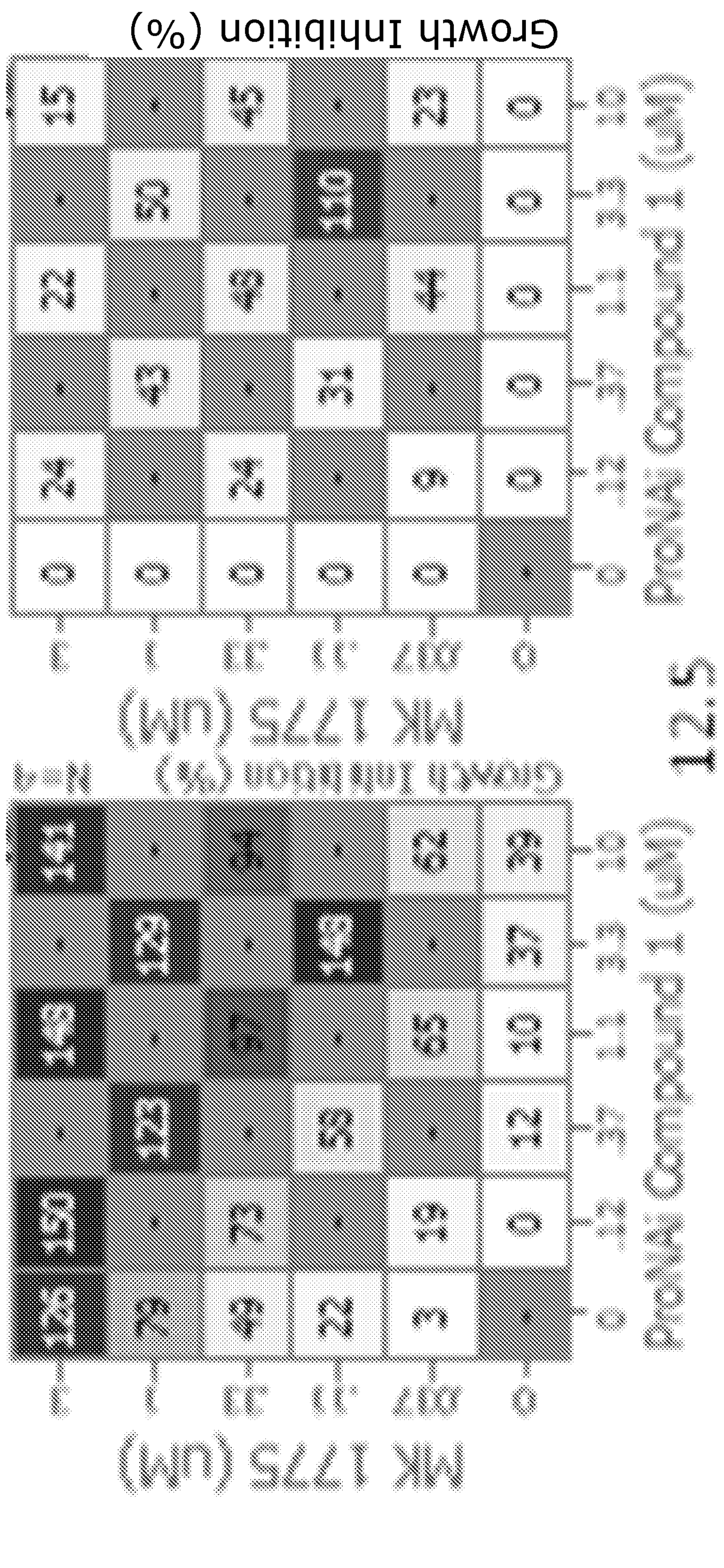


FIG 6 (Cont'd)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/26954

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/501 (2018.01)
CPC - A61K 2300/00, A61K 31/5025

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)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2015/0126471 A1 (Cancer Research Technology Limited) 07 May 2015 (07.05.2015); para [0011], [0030], [0035], [0039], [0051], [0120], [0144], [0178], [0189], [0204]-[0205]	1-5, 18-21, 27, 29/27, 30-32 ---------- 28, 29/28
X -- Y	WO 2014/062454 A1 (MERCK SHARP & DOHME CORP.) 24 April 2014 (24.04.2014); pg. 8, ln 20-21, pg. 10, ln 16-20, pg. 12, ln 35-38, pg. 15, ln 10-14, pg. 18, ln 23-25, pg. 27, ln 28-30, pg. 51, ln 7-14	34-41 ---------- 28, 29/28
A	US 2005/0250836A1 (Booth et al.) 10 November 2005 (10.11.2005); entire document	1-5, 18-21, 27-32, 34-41
A	WO 2012/074754 A1 (ARRAY BIOPHARMA INC.) 07 June 2012 (07.06.2012); entire document	1-5, 18-21, 27-32, 34-41

☐ 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	Date of mailing of the international search report
12 June 2018	28 JUN 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/26954

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-17, 22-26, 33, 42-50
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.