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(54) **Title:** MODULATORS OF PYRIMIDINE NUCLEOTIDE BIOSYNTHETIC PATHWAYS

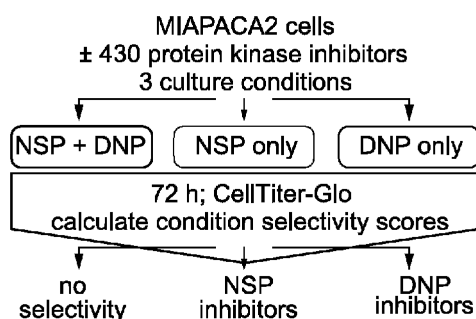

FIG. 1A

(57) **Abstract:** The disclosure is directed, *inter alia*, to compounds and compositions that modulate pyrimidine nucleotide biosynthetic pathways, such as the pyrimidine nucleoside salvage pathway and the pyrimidine de novo pathway, and to their use in treating cancer.

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MODULATORS OF PYRIMIDINE NUCLEOTIDE BIOSYNTHETIC PATHWAYS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US Application No. 62/848,728 filed May 16, 2019, the disclosure of which is incorporated by reference herein in its entirety.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with government support under Grant Number CA187678, awarded by The National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] The redundant and plastic nature of metabolic networks represents a significant obstacle in the targeting of cancer metabolism. This hurdle manifests in two ways, the first being redundancy in enzymes performing identical biochemical reactions, such as the hexokinase isozymes which phosphorylate glucose (Xu et al., 2018). The second is the presence of convergent, parallel metabolic pathways producing a common metabolite from unique precursors, usually consisting of salvage and *de novo* arms. Such convergent metabolic nodes have been noted in nucleotide (Le et al., 2017), lipid (cholesterol)(York et al., 2015) and amino acid (aspartate) metabolism (Garcia-Bermudez et al., 2018).

[0004] Despite these difficulties, the development of metabolism modifiers remains a robust area of research. One such therapeutically relevant target is pyrimidine nucleotide biosynthesis which consists of nucleoside salvage (NSP) and *de novo* (DNP) pathways which converge to generate uridine monophosphate (UMP), the common precursor for all pyrimidine nucleotides (Okesli et al., 2017). The NSP scavenges preformed nucleosides from the extracellular environment, shuttling them into the cell via nucleoside transporters where they are phosphorylated by uridine-cytidine kinases (UCKs) to produce UMP. UCK2 is thought to be the primary NSP kinase, given its 20-fold higher catalytic efficiency compared with UCK1 (Van Rompay et al., 2001). The DNP consists of a six-step process that utilizes glutamine, aspartate, bicarbonate and glucose to produce UMP through the action of the trifunctional enzyme CAD, electron transport chain-linked dihydroorotate dehydrogenase (DHODH), and bifunctional UMP synthase (UMPS). Amongst these proteins, DHODH in particular has been the subject of significant research interest in anticancer settings (Madak et al., 2019; Sykes et al., 2016;

Santana-Codina et al., 2018; Lolli et al., 2018).

[0005] The disclosure is directed, *inter alia*, to the exploitation of nucleotide biosynthetic pathways in treating cancer and to other important ends.

BRIEF SUMMARY

[0006] The disclosure provides methods for identifying compounds that inhibit cancer cell proliferation by: (i) contacting the compounds with (a) cancer cells and salvage pathway condition-specific growth media and de novo pathway condition-specific growth media; and/or (b) cancer cells and salvage pathway condition-specific growth media; and/or (c) cancer cells and de novo pathway condition-specific growth media; wherein the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite; and (ii) identifying inhibition of cancer cell proliferation by the compound; thereby identifying the compound that inhibits cancer cell proliferation. In aspects, the salvage pathway is a pyrimidine nucleoside salvage pathway, the de novo pathway is de novo nucleotide pathway, and the common metabolite is uridine monophosphate (UMP).

[0007] The disclosure provides methods of treating cancer in patients in need thereof using compounds that inhibit cancer cell proliferation, wherein the compounds are identified and selected by (i) contacting the compounds with (a) cancer cells and salvage pathway condition-specific growth media and de novo pathway condition-specific growth media; and/or (b) cancer cells and salvage pathway condition-specific growth media; and/or (c) cancer cells and de novo pathway condition-specific growth media; wherein the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite; (ii) identifying inhibition of cancer cell proliferation by the compound; thereby identifying the compound that inhibits cancer cell proliferation; (iii) administering the compound that inhibits cancer cell proliferation to a patient in need thereof. In aspects, the salvage pathway is a pyrimidine nucleoside salvage pathway, the de novo pathway is de novo nucleotide pathway, and the common metabolite is uridine monophosphate (UMP). In aspects, the cancer is pancreatic cancer, such as pancreatic ductal adenocarcinoma.

[0008] The disclosure provides methods of treating cancer in patients in need thereof by administering a therapeutically effective amount of OSU-03012, TAK-632, JNK-IN-8, CNX-774, or motesanib. In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma.

[0009] The disclosure provides compositions comprising: (i) dihydroorotate dehydrogenase and (ii) TAK-632 or OSU-03012. In aspects, the composition is a complex. In aspects, the

composition is a co-crystal. In aspects, (i) and (ii) are bonded together via one or more hydrogen bonds.

[0010] Other embodiments and aspects of the disclosure are described in more detail herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIGS. 1A-1F provide the identification of UMP-DNP and -NSP modulators in a small molecule protein kinase inhibitor library. FIG. 1A provides the phenotypic screening strategy, where the impact of 430 protein kinase inhibitors on cell proliferation was evaluated in MIAPACA2 cells plated in 3 distinct growth conditions: (i) NSP + DNP (media + 10 μM uridine (rU)); (ii) NSP only (media + 10 μM rU + 1 μM NITD-982); or (iii) DNP only (media alone). % proliferation values were calculated using CellTiter-Glo (CTG) following 72 hours treatment (7-point dose response; n=2). FIGS. 1B and 1C are waterfall plots ranking library compounds based on NSP (FIG. 1B) or DNP (FIG. 1C) pathway selectivity scores determined as described in Fig. S3B. FIGS. 1D-1F provide a summary of NSP and DNP selectivity scores across library compounds annotated as JNK (FIG. 1D), PDK1 inhibitors (FIG. 1E), or RAF inhibitors (FIG. 1F).

[0012] FIGS. 2A-2D show that JNK-IN-8 inhibits nucleoside transport *in vitro* and *in vivo*. FIG. 2A shows that nucleoside uptake can be prevented by inhibition of either nucleoside transporters or kinases. FIG. 2B shows the uptake of ^{3}H-rU or ^{3}H-dC in CCRF-CEM cells following 2 hours incubation ± JNK-IN-8 (mean±SD; n=2). IC_{50} values are indicated. FIG. 2C provides representative PET/CT scans of CCRF-CEM subcutaneous tumor bearing NSG mice imaged with [^{18}F]CFA 4 h after treatment with vehicle (n=2) or JNK-IN-8 (50 mg/kg; n=3) T, tumor. FIG. 2D shows the quantification of [^{18}F]CFA tumor uptake in FIG. 2C (%ID/g: percentage injected dose per gram; mean±SD; student t-test, * $P<0.05$).

[0013] FIGS. 3A-3H show that OSU-03012 and TAK-632 inhibit DHODH and activate the DNA replication stress response pathway. FIG. 3A shows the UMP biosynthesis via the *de novo* and salvage pathways. FIG. 3B provides a propidium iodide cell cycle analysis of MIAPACA2 PDAC cells treated ±5 μM TAK-632 or ±5 μM OSU-03012 and supplemented with 50 μM orotate (OA) or 10 μM rU (N.S.: no supplement). Insert indicates % S-phase cells. FIG. 3C shows a summary of fold changes in S-phase cells from B (mean±SD; n=2; one-way ANOVA corrected for multiple comparisons by Bonferroni adjustment, ns: not significant; * $P<0.05$; ** $P<0.01$). FIG. 3D provides an *in vitro* DHODH enzyme assay performed in the presence of OSU-03012 or TAK-632. FIG. 3E provides a correlation between DHODH inhibitor (1 μM NITD-982) and OSU-03012 (3.17 μM) or TAK-632 (3.17 μM) response across a panel of 25

PDAC cell lines determined using CTG following 72 h treatment. Response calculated as doubling time normalized proliferation inhibition. Pearson correlation coefficient is indicated. FIG. 3F provides an immunoblot analysis of MIAPACA2 cells treated ±1 μM PDK1 inhibitor GSK-2334470 (GSK) ±1 μM OSU-03012 (OSU) ±10 μM rU for 24 h. FIG. 3G is an immunoblot analysis of MIAPACA2 cells treated ±1 μM RAF inhibitor LY3009120 (LY) ±5 μM TAK-632 (TAK) ±10 μM rU for 24 h. FIG. 3H is an annexin V/PI flow cytometry analysis of MIAPACA2 PDAC cells treated with 1 μM OSU-03012 or 1 μM GSK-2334470 (GSK) ±500 nM M6620 (ATRi) ±25 μM rU for 72 h (mean±SD; n=2; one-way ANOVA corrected for multiple comparisons by Bonferroni adjustment; ns: not significant; ** P<0.01; *** P<0.001).

[0014] FIGS. 4A-4B show that OSU-03012 and TAK-632 bind DHODH and show the crystal structure of DHODH with OSU-03012 (FIG. 4A) or TAK-632 (FIG. 4B). 2mFo-DFc electron density for OSU-03012 (carbons in yellow) or TAK-632 (carbons in green) contoured at 1 σ. Dashed black lines represent hydrogen bonds between the ligands and DHODH. Interacting residues as predicted by LigPlot^{+} are shown and labeled.

[0015] FIGS. 5A-5B provide validation of UMP as a critical, convergent metabolic node in cancer cells. FIG. 5A shows that UMP can be produced by a *de novo* pathway (DNP) from glucose, glutamine, bicarbonate and aspartate or from extracellular uridine (rU) by a nucleoside transporter and kinase-dependent salvage pathway (NSP). FIG. 5B provides dose response curves of DHODH inhibitor NITD-982 and nucleoside transport inhibitor dipyridamole (DPA) in JURKAT cells cultured in NSP + DNP (media + 10 μM rU), NSP only (media + 10 μM rU + 1 μM NITD-982), or DNP only (media alone) for 72 h as determined by CellTiter-Glo (CTG; mean±SD; n=4).

[0016] FIG. 6 shows that UMP-DNP and NSP are interchangeable in sustaining proliferation across a panel of cancer cell lines. Uridine titration in cancer cell lines cultured in media + 10% dFBS ±1 μM NITD-982 (n=4). Relative proliferation rate (PR) was calculated by normalizing % proliferation values at 72 h to cell line proliferation rate. Proliferation rates were calculated utilizing CTG measurements at the time of treatment (t0) and vehicle-treated controls at 72 h.

[0017] FIGS. 7A-7F shows a phenotypic screen identifies UMP-NSP and -DNP inhibitors. FIG. 7A provides a CTG analysis of MIAPACA2 cells cultured in NSP+DNP (media +10 μM rU), DNP (media alone), NSP (media +10 μM rU +1 μM NITD-982) or starvation conditions (media + 1 μM NITD-982) for 72 h (mean±SD; n=4; one-way ANOVA corrected for multiple comparisons by Bonferroni adjustment, ** P < 0.01; *** P < 0.001). FIG. 7B shows the methodology applied to determine UMP-DNP and -NSP selectivity scores (n=2). FIG. 7C shows

Z'-scores calculated for individual assay plates from experiment in FIG. 1. FIG. 7D shows selectivity scores for BTK inhibitors included in the screen. FIG. 7E shows selectivity scores for VEGFR inhibitors included in the screen, where the hits included CNX-774 and motesanib. FIG. 7F shows validation of hit compounds using 7 d crystal violet proliferation assay. MIAPACA2 cells were treated with 1 µM JNK-IN-8, 1 µM OSU-03012, 1 µM TAK-632 in NSP+DNP, DNP or NSP conditions.

[0018] FIGS. 8A-8C summarize the evaluation of UMP-NSP and -DNP inhibitor potency and selectivity. FIG. 8A shows calculation of JNK-IN-8, CNX-774, and motesanib IC50 values for JURKAT cells cultured in NSP+DNP, DNP-only, and NSP-only conditions, and EC50 values for gemcitabine (dFdC) rescue. FIG. 8B shows JNK-IN-8 and DPA dose response in JURKAT cells treated with 10 nM dFdC (n=4; mean±SD) for 72 h determined using CTG. FIG. 8C shows calculation of OSU-03012 and TAK-632 IC50 in JURKAT cells treated for 72 h determined using CTG.

[0019] FIGS. 9A-9C shows the evaluation of DHODH / inhibitor interactions. FIG. 9A provides the crystallographic data collection and refinement statistics. A high resolution shell in parenthesis; r.m.s., rootmean-square; a.u., asymmetric unit. FIG. 9B provides a stereoscopic image of FIG. 4A (OSU-03012). FIG. 9C provides a stereoscopic image of FIG. 4B (TAK-632).

[0020] FIG. 10 shows the annexinV/PI flow cytometry analysis of OSU-03012/ATRi combination. Representative flow cytometry plots from experiment in FIG. 3H.

DETAILED DESCRIPTION

[0021] Definitions

[0022] "Pyrimidine nucleotide biosynthesis" refers to the nucleoside salvage pathway (NSP) and the *de novo* (DNP) pathway which converge to generate uridine monophosphate (UMP), the common precursor for all pyrimidine nucleotides (Okesli et al., 2017).

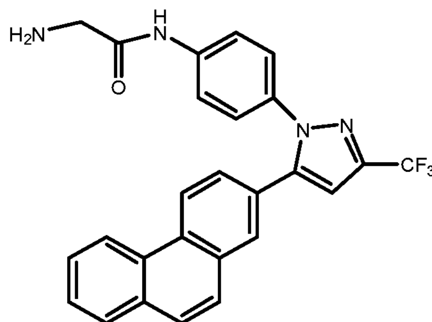
[0023] "Nucleoside salvage pathway" and "NSP" and "pyrimidine nucleoside salvage pathway" salvages preformed nucleosides from the extracellular environment, shuttling them into the cell via nucleoside transporters where they are phosphorylated by uridine-cytidine kinases (UCKs) to produce UMP.

[0024] "De novo pathway" and "DNP" and "de novo nucleotide pathway" consists of a six-step process that utilizes glutamine, aspartate, bicarbonate and glucose to produce UMP through the action of the trifunctional enzyme CAD, electron transport chain-linked dihydroorotate dehydrogenase (DHODH), and bifunctional uridine monophosphate synthase (UMPS).

[0025] "Dihydroorotate dehydrogenase" and "DHODH" and "DHOD" refer to a protein which catalyzes the fourth step in the de novo pyrimidine nucleotide pathway. DHODH catalyzes the only oxidation/reduction reaction in that pathway which is the step of converting dihydroorotate to orotate with the aid of flavin cofactor and an electron acceptor.

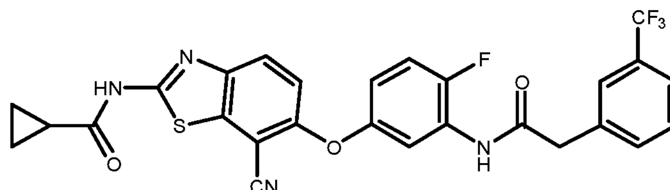
[0026] "Condition-specific growth media" refers to in vitro growth media that will allow cancer cells to grow under any given conditions. In embodiments, the condition-specific growth media allows cancer cells to grow under salvage pathway conditions and/or do novo pathway conditions, wherein the salvage pathway and the de novo pathway are convergent metabolic pathways that produce at least one common metabolite. In embodiments, the condition-specific growth media allows cancer cells to grow under pyrimidine nucleoside salvage pathway conditions and de novo nucleotide pathway conditions, wherein these convergent metabolic pathways produce uridine monophosphate as the common metabolite. Condition-specific growth media can readily be determined by the skilled artisan based on the specific cancer cells and specific convergent metabolic pathways being evaluated. In aspects, the cancer cells are pancreatic cancer cells.

[0027] "OSU-03012" or "AR-12" or "2-amino-*N*-[4-[5-phenanthren-2-yl-3-(trifluoromethyl)-pyrazol-1-yl]phenyl]acetamide" refers to a compound having the structure:



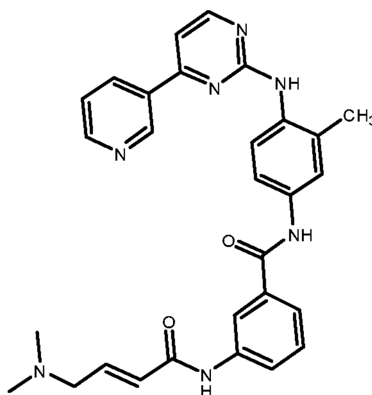
Methods for making OSU-03012 are described in US Patent No. 8,039,502, the disclosure of which is incorporated by reference herein in its entirety.

[0028] "TAK-632" or "N-(7-cyano-6-(4-fluoro-3-(2-(3-(trifluoromethyl)phenyl)acetamido)-phenoxy)benzo[d]thiazol-2-yl)cyclopropanecarboxamide" refers to a compound having the structure:

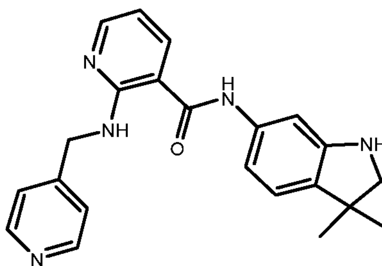


Methods for making TAK-632 are described in US Patent No. 8,143,258, the disclosure of which is incorporated by reference herein in its entirety.

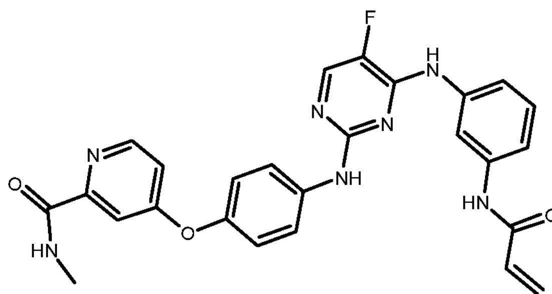
[0029] "JNK-IN-8" or "3-[[4-(dimethylamino)-1-oxo-2-buten-1-yl]amino]-*N*-[3-methyl-4-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]benzamide" refers to a compound having the structure:



[0030] "CNX-774" or "4-[4-[[5-fluoro-4-[3-(prop-2-enoylamino)anilino]pyrimidin-2-yl]amino]phenoxy]-N-methylpyridine-2-carboxamide" refers to a compound having the structure:



[0031] "Motesanib" and "AMG-706" refer to a compound having the structure:



[0032] The terms "a" or "an," as used in herein means one or more. In addition, the phrase "substituted with a[n]," as used herein, means the specified group may be substituted with one or more of any or all of the named substituents. For example, where a group, such as an alkyl or heteroaryl group, is "substituted with an unsubstituted C_1-C_{20} alkyl, or unsubstituted 2 to 20

membered heteroalkyl," the group may contain one or more unsubstituted C_1-C_{20} alkyls, and/or one or more unsubstituted 2 to 20 membered heteroalkyls.

[0033] A “detectable agent” or “detectable moiety” is a compound or composition detectable by appropriate means such as spectroscopic, photochemical, biochemical, immunochemical, chemical, magnetic resonance imaging, or other physical means. For example, useful detectable agents include ^{18}F, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{77}As, ^{86}Y, ^{90}Y. ^{89}Sr, ^{89}Zr, ^{94}Tc, ^{94}Tc, ^{99m}Tc, ^{99}Mo, ^{105}Pd, ^{105}Rh, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, $^{154-1581}$Gd, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac, Cr, V, Mn, Fe, Co, Ni, Cu, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, ^{32}P, fluorophore (e.g. fluorescent dyes), electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, paramagnetic molecules, paramagnetic nanoparticles, ultrasmall superparamagnetic iron oxide nanoparticles, USPIO nanoparticle aggregates, superparamagnetic iron oxide nanoparticles, SPIO nanoparticle aggregates, monocrystalline iron oxide nanoparticles, monocrystalline iron oxide, nanoparticle contrast agents, liposomes or other delivery vehicles containing Gadolinium chelate molecules, Gadolinium, radioisotopes, radionuclides (e.g. carbon-11, nitrogen-13, oxygen-15, fluorine-18, rubidium-82), fluorodeoxyglucose (e.g. fluorine-18 labeled), any gamma ray emitting radionuclides, positron-emitting radionuclide, radiolabeled glucose, radiolabeled water, radiolabeled ammonia, biocolloids, microbubbles (e.g. including microbubble shells including albumin, galactose, lipid, and/or polymers; microbubble gas core including air, heavy gas(es), perfluorcarbon, nitrogen, octafluoropropane, perflexane lipid microsphere, perflutren, etc.), iodinated contrast agents (e.g. iohexol, iodixanol, ioversol, iopamidol, ioxilan, iopromide, diatrizoate, metrizoate, ioxaglate), barium sulfate, thorium dioxide, gold, gold nanoparticles, gold nanoparticle aggregates, fluorophores, two-photon fluorophores, or haptens and proteins or other entities which can be made detectable, e.g., by incorporating a radiolabel into a peptide or antibody specifically reactive with a target peptide. A detectable moiety is a monovalent detectable agent or a detectable agent capable of forming a bond with another composition.

[0034] Radioactive substances (e.g., radioisotopes) that may be used as imaging and/or labeling agents in accordance with the embodiments of the disclosure include, but are not limited to, ^{18}F, ^{32}P, ^{33}P, ^{45}Ti, ^{47}Sc, ^{52}Fe, ^{59}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{77}As, ^{86}Y, ^{90}Y. ^{89}Sr, ^{89}Zr, ^{94}Tc, ^{94}Tc, ^{99m}Tc, ^{99}Mo, ^{105}Pd, ^{105}Rh, ^{111}Ag, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{153}Sm, $^{154-1581}$Gd, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{169}Er, ^{175}Lu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{194}Ir, ^{198}Au, ^{199}Au, ^{211}At, ^{211}Pb, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra and ^{225}Ac. Paramagnetic ions that may be used as

additional imaging agents in accordance with the embodiments of the disclosure include, but are not limited to, ions of transition and lanthanide metals (e.g. metals having atomic numbers of 21-29, 42, 43, 44, or 57-71). These metals include ions of Cr, V, Mn, Fe, Co, Ni, Cu, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu.

[0035] As used herein, the term "cancer" refers to all types of cancer, neoplasm or malignant tumors found in mammals (e.g. humans), including leukemias, lymphomas, carcinomas and sarcomas. Exemplary cancers that may be treated with a compound or method provided herein include pancreatic cancer (e.g., pancreatic ductal adenocarcinoma), brain cancer, glioma, glioblastoma, neuroblastoma, prostate cancer, colorectal cancer, Medulloblastoma, melanoma, cervical cancer, gastric cancer, ovarian cancer, lung cancer, cancer of the head, Hodgkin's Disease, and Non-Hodgkin's Lymphomas. Exemplary cancers that may be treated with a compound or method provided herein include cancer of the thyroid, endocrine system, brain, breast, cervix, colon, head & neck, liver, kidney, lung, ovary, pancreas, rectum, stomach, and uterus. Additional examples include, thyroid carcinoma, cholangiocarcinoma, pancreatic cancer, pancreatic adenocarcinoma, pancreatic ductal adenocarcinoma, skin cutaneous melanoma, colon adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, breast invasive carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, non-small cell lung carcinoma, mesothelioma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer, hepatocellular carcinoma, or prostate cancer.

[0036] A “cell” as used herein, refers to a cell carrying out metabolic or other function sufficient to preserve or replicate its genomic DNA. A cell can be identified by well-known methods in the art including, for example, presence of an intact membrane, staining by a particular dye, ability to produce progeny or, in the case of a gamete, ability to combine with a second gamete to produce a viable offspring. Cells may include prokaryotic and eukaroytic cells. Prokaryotic cells include but are not limited to bacteria. Eukaryotic cells include but are not limited to yeast cells and cells derived from plants and animals, for example mammalian, insect (*e.g.*, spodoptera) and human cells. Cells may be useful when they are naturally nonadherent or have been treated not to adhere to surfaces, for example by trypsinization. Cells may include

cancer cells.

[0037] "Control" or "control experiment" is used in accordance with its plain ordinary meaning and refers to an experiment in which the subjects or reagents of the experiment are treated as in a parallel experiment except for omission of a procedure, reagent, or variable of the experiment. In some instances, the control is used as a standard of comparison in evaluating experimental effects. In aspects, a control is the measurement of the growth, activity, or function of cancer cells in the absence of a compound as described herein (including embodiments, aspects, and examples).

[0038] Cancer model organism, as used herein, is an organism exhibiting a phenotype indicative of cancer, or the activity of cancer causing elements, within the organism. The term cancer is defined above. A wide variety of organisms may serve as cancer model organisms, and include for example, cancer cells and mammalian organisms such as rodents (e.g. mouse or rat) and primates (such as humans). Cancer cell lines are widely understood by those skilled in the art as cells exhibiting phenotypes or genotypes similar to in vivo cancers. Cancer cell lines as used herein includes cell lines from animals (e.g. mice) and from humans.

[0039] An "anticancer agent" as used herein refers to a molecule (e.g. compound, peptide, protein, nucleic acid) used to treat cancer through destruction or inhibition of cancer cells or tissues. Anticancer agents may be selective for certain cancers or certain tissues.

[0040] "Anti-cancer agent" and "anticancer agent" are used in accordance with their plain ordinary meaning and refers to a composition (e.g. compound, drug, antagonist, inhibitor, modulator) having antineoplastic properties or the ability to inhibit the growth or proliferation of cells. In aspects, an anti-cancer agent is a chemotherapeutic. In aspects, an anti-cancer agent is an agent identified herein having utility in methods of treating cancer. In aspects, an anti-cancer agent is an agent approved by the FDA or similar regulatory agency of a country other than the USA, for treating cancer. Examples of anti-cancer agents include, but are not limited to, MEK (e.g. MEK1, MEK2, or MEK1 and MEK2) inhibitors (e.g. XL518, CI-1040, PD035901, selumetinib/ AZD6244, GSK1120212/ trametinib, GDC-0973, ARRY-162, ARRY-300, AZD8330, PD0325901, U0126, PD98059, TAK-733, PD318088, AS703026, BAY 869766), alkylating agents (e.g., cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, nitrogen mustards (e.g., mechloroethamine, cyclophosphamide, chlorambucil, meiphalan), ethylenimine and methylmelamines (e.g., hexamethlymelamine, thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine, lomusitne, semustine, streptozocin), triazenes (decarbazine)),

anti-metabolites (e.g., 5- azathioprine, leucovorin, capecitabine, fludarabine, gemcitabine, pemetrexed, raltitrexed, folic acid analog (e.g., methotrexate), or pyrimidine analogs (e.g., fluorouracil, floxouridine, Cytarabine), purine analogs (e.g., mercaptopurine, thioguanine, pentostatin), etc.), plant alkaloids (e.g., vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, docetaxel, etc.), topoisomerase inhibitors (e.g., irinotecan, topotecan, amsacrine, etoposide (VP16), etoposide phosphate, teniposide, etc.), antitumor antibiotics (e.g., doxorubicin, adriamycin, daunorubicin, epirubicin, actinomycin, bleomycin, mitomycin, mitoxantrone, plicamycin, etc.), platinum-based compounds (e.g. cisplatin, oxaloplatin, carboplatin), anthracenedione (e.g., mitoxantrone), substituted urea (e.g., hydroxyurea), methyl hydrazine derivative (e.g., procarbazine), adrenocortical suppressant (e.g., mitotane, aminoglutethimide), epipodophyllotoxins (e.g., etoposide), antibiotics (e.g., daunorubicin, doxorubicin, bleomycin), enzymes (e.g., L-asparaginase), inhibitors of mitogen-activated protein kinase signaling (e.g. U0126, PD98059, PD184352, PD0325901, ARRY-142886, SB239063, SP600125, BAY 43-9006, wortmannin, or LY294002, Syk inhibitors, mTOR inhibitors, antibodies (e.g., rituxan), gossyphol, genasense, polyphenol E, Chlorofusin, all trans-retinoic acid (ATRA), bryostatin, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), 5-aza-2'-deoxycytidine, all trans retinoic acid, doxorubicin, vincristine, etoposide, gemcitabine, imatinib (Gleevec.RTM.), geldanamycin, 17-N-Allylamino-17-Demethoxygeldanamycin (17-AAG), flavopiridol, LY294002, bortezomib, trastuzumab, BAY 11-7082, PKC412, PD184352, 20-epi-1, 25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline

sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; 9-dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide

antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylerie conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen-binding protein; sizofuran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin stimalamer, Adriamycin, Dactinomycin, Bleomycin, Vinblastine, Cisplatin, acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate;

aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; iimofosine; interleukin I1 (including recombinant interleukin II, or rlL.sub.2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-1a; interferon gamma-1b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazoie; nogalamycin; ormaplatin; oxisuran; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin

hydrochloride, agents that arrest cells in the G2-M phases and/or modulate the formation or stability of microtubules, (e.g. Taxol.TM (i.e. paclitaxel), Taxotere.TM, compounds comprising the taxane skeleton, Erbulozole (i.e. R-55104), Dolastatin 10 (i.e. DLS-10 and NSC-376128), Mivobulin isethionate (i.e. as CI-980), Vincristine, NSC-639829, Discodermolide (i.e. as NVP-XX-A-296), ABT-751 (Abbott, i.e. E-7010), Altorhyrtins (e.g. Altorhyrtin A and Altorhyrtin C), Spongistatins (e.g. Spongistatin 1, Spongistatin 2, Spongistatin 3, Spongistatin 4, Spongistatin 5, Spongistatin 6, Spongistatin 7, Spongistatin 8, and Spongistatin 9), Cemadotin hydrochloride (i.e. LU-103793 and NSC-D-669356), Epothilones (e.g. Epothilone A, Epothilone B, Epothilone C (i.e. desoxyepothilone A or dEpoA), Epothilone D (i.e. KOS-862, dEpoB, and desoxyepothilone B), Epothilone E, Epothilone F, Epothilone B N-oxide, Epothilone A N-oxide, 16-aza-epothilone B, 21-aminoepothilone B (i.e. BMS-310705), 21-hydroxyepothilone D (i.e. Desoxyepothilone F and dEpoF), 26-fluoroepothilone, Auristatin PE (i.e. NSC-654663), Soblidotin (i.e. TZT-1027), LS-4559-P (Pharmacia, i.e. LS-4577), LS-4578 (Pharmacia, i.e. LS-477-P), LS-4477 (Pharmacia), LS-4559 (Pharmacia), RPR-112378 (Aventis), Vincristine sulfate, DZ-3358 (Daiichi), FR-182877 (Fujisawa, i.e. WS-9885B), GS-164 (Takeda), GS-198 (Takeda), KAR-2 (Hungarian Academy of Sciences), BSF-223651 (BASF, i.e. ILX-651 and LU-223651), SAH-49960 (Lilly/Novartis), SDZ-268970 (Lilly/Novartis), AM-97 (Armad/Kyowa Hakko), AM-132 (Armad), AM-138 (Armad/Kyowa Hakko), IDN-5005 (Indena), Cryptophycin 52 (i.e. LY-355703), AC-7739 (Ajinomoto, i.e. AVE-8063A and CS-39.HCl), AC-7700 (Ajinomoto, i.e. AVE-8062, AVE-8062A, CS-39-L-Ser.HCl, and RPR-258062A), Vitilevuamide, Tubulysin A, Canadensol, Centaureidin (i.e. NSC-106969), T-138067 (Tularik, i.e. T-67, TL-138067 and TI-138067), COBRA-1 (Parker Hughes Institute, i.e. DDE-261 and WHI-261), H10 (Kansas State University), H16 (Kansas State University), Oncocidin A1 (i.e. BTO-956 and DIME), DDE-313 (Parker Hughes Institute), Fijianolide B, Laulimalide, SPA-2 (Parker Hughes Institute), SPA-1 (Parker Hughes Institute, i.e. SPIKET-P), 3-IAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-569), Narcosine (also known as NSC-5366), Nascapine, D-24851 (Asta Medica), A-105972 (Abbott), Hemiasterlin, 3-BAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-191), TMPN (Arizona State University), Vanadocene acetylacetonate, T-138026 (Tularik), Monsatrol, lnanocine (i.e. NSC-698666), 3-IAABE (Cytoskeleton/Mt. Sinai School of Medicine), A-204197 (Abbott), T-607 (Tuiarik, i.e. T-900607), RPR-115781 (Aventis), Eleutherobins (such as Desmethyleleutherobin, Desaetyleleutherobin, lsoeleutherobin A, and Z-Eleutherobin), Caribaeoside, Caribaeolin, Halichondrin B, D-64131 (Asta Medica), D-68144 (Asta Medica), Diazonamide A, A-293620 (Abbott), NPI-2350 (Nereus), Taccalonolide A, TUB-245 (Aventis), A-259754 (Abbott),

Diozostatin, (-)-Phenylahistin (i.e. NSCL-96F037), D-68838 (Asta Medica), D-68836 (Asta Medica), Myoseverin B, D-43411 (Zentaris, i.e. D-81862), A-289099 (Abbott), A-318315 (Abbott), HTI-286 (i.e. SPA-110, trifluoroacetate salt) (Wyeth), D-82317 (Zentaris), D-82318 (Zentaris), SC-12983 (NCI), Resverastatin phosphate sodium, BPR-OY-007 (National Health Research Institutes), and SSR-250411 (Sanofi)), steroids (e.g., dexamethasone), finasteride, aromatase inhibitors, gonadotropin-releasing hormone agonists (GnRH) such as goserelin or leuprolide, adrenocorticosteroids (e.g., prednisone), progestins (e.g., hydroxyprogesterone caproate, megestrol acetate, medroxyprogesterone acetate), estrogens (e.g., diethlystilbestrol, ethinyl estradiol), antiestrogen (e.g., tamoxifen), androgens (e.g., testosterone propionate, fluoxymesterone), antiandrogen (e.g., flutamide), immunostimulants (e.g., Bacillus Calmette-Guérin (BCG), levamisole, interleukin-2, alpha-interferon, etc.), monoclonal antibodies (e.g., anti-CD20, anti-HER2, anti-CD52, anti-HLA-DR, and anti-VEGF monoclonal antibodies), immunotoxins (e.g., anti-CD33 monoclonal antibody-calicheamicin conjugate, anti-CD22 monoclonal antibody-pseudomonas exotoxin conjugate, etc.), radioimmunotherapy (e.g., anti-CD20 monoclonal antibody conjugated to ^{111}In, ^{90}Y, or ^{131}I, etc.), triptolide, homoharringtonine, dactinomycin, doxorubicin, epirubicin, topotecan, itraconazole, vindesine, cerivastatin, vincristine, deoxyadenosine, sertraline, pitavastatin, irinotecan, clofazimine, 5-nonyloxytryptamine, vemurafenib, dabrafenib, erlotinib, gefitinib, EGFR inhibitors, epidermal growth factor receptor (EGFR)-targeted therapy or therapeutic (e.g. gefitinib (Iressa ™), erlotinib (Tarceva ™), cetuximab (Erbitux™), lapatinib (Tykerb™), panitumumab (Vectibix™), vandetanib (Caprelsa™), afatinib/BIBW2992, CI-1033/canertinib, neratinib/HKI-272, CP-724714, TAK-285, AST-1306, ARRY334543, ARRY-380, AG-1478, dacomitinib/PF299804, OSI-420/desmethyl erlotinib, AZD8931, AEE788, pelitinib/EKB-569, CUDC-101, WZ8040, WZ4002, WZ3146, AG-490, XL647, PD153035, BMS-599626), sorafenib, imatinib, sunitinib, dasatinib, or the like.

[0041] "Selective" or "selectivity" or the like of a compound refers to the compound's ability to discriminate between molecular targets (e.g., a compound having selectivity for one metabolic pathway over another metabolic pathway).

[0042] "Specific", "specifically", "specificity", or the like of a compound refers to the compound's ability to cause a particular action, such as inhibition, to a particular molecular target or metabolic pathway with minimal or no action to other molecular targets or metabolic pathways.

[0043] The term "pharmaceutically acceptable salts" is meant to include salts of the active

compounds that are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the disclosure contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the disclosure contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogen carbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogen sulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, oxalic, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge et al., "Pharmaceutical Salts", Journal of Pharmaceutical Science, 1977, 66, 1-19). Certain specific compounds of the disclosure contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

[0044] Thus, the compounds of the disclosure may exist as salts, such as with pharmaceutically acceptable acids. The disclosure includes such salts. Non-limiting examples of such salts include hydrochlorides, hydrobromides, phosphates, sulfates, methanesulfonates, nitrates, maleates, acetates, citrates, fumarates, proprionates, tartrates (e.g., (+)-tartrates, (-)-tartrates, or mixtures thereof including racemic mixtures), succinates, benzoates, and salts with amino acids such as glutamic acid, and quaternary ammonium salts (e.g. methyl iodide, ethyl iodide, and the like). These salts may be prepared by methods known to those skilled in the art. The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound may differ from the various salt forms in certain physical properties, such as solubility in polar solvents. In addition to salt forms, the disclosure provides compounds, which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the disclosure. Prodrugs of the compounds described herein may be converted *in vivo* after

administration. Additionally, prodrugs can be converted to the compounds of the disclosure by chemical or biochemical methods in an *ex vivo* environment, such as, for example, when contacted with a suitable enzyme or chemical reagent. Certain compounds of the disclosure can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the disclosure. Certain compounds of the disclosure may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the disclosure and are intended to be within the scope of the disclosure.

[0045] As used herein, the term "about" means a range of values including the specified value, which a person of ordinary skill in the art would consider reasonably similar to the specified value. In aspects, about means within a standard deviation using measurements generally acceptable in the art. In aspects, about means a range extending to +/- 10% of the specified value. In aspects, about includes the specified value.

[0046] The terms "treating", or "treatment" refers to any indicia of success in the therapy or amelioration of an injury, disease, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a patient's physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation. The term "treating" and conjugations thereof, may include prevention of an injury, pathology, condition, or disease. In aspects, treating is preventing. In aspects, treating does not include preventing.

[0047] "Treating" or "treatment" as used herein (and as well-understood in the art) also broadly includes any approach for obtaining beneficial or desired results in a subject's condition, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of the extent of a disease, stabilizing (*i.e.*, not worsening) the state of disease, prevention of a disease's transmission or spread, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission, whether partial or total and whether detectable or undetectable. In other words, "treatment" as used herein includes any cure, amelioration, or prevention of a disease. Treatment may prevent the disease from occurring; inhibit the disease's spread; relieve the disease's symptoms, fully or partially remove

the disease's underlying cause, shorten a disease's duration, or do a combination of these things.

[0048] "Treating" and "treatment" as used herein include prophylactic treatment. Treatment methods include administering to a subject a therapeutically effective amount of an active agent. The administering step may consist of a single administration or may include a series of administrations. The length of the treatment period depends on a variety of factors, such as the severity of the condition, the age of the patient, the concentration of active agent, the activity of the compositions used in the treatment, or a combination thereof. It will also be appreciated that the effective dosage of an agent used for the treatment or prophylaxis may increase or decrease over the course of a particular treatment or prophylaxis regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic administration may be required. For example, the compositions are administered to the subject in an amount and for a duration sufficient to treat the patient. In aspects, the treating or treatment is no prophylactic treatment.

[0049] The term "prevent" refers to a decrease in the occurrence of disease symptoms in a patient. As indicated above, the prevention may be complete (no detectable symptoms) or partial, such that fewer symptoms are observed than would likely occur absent treatment.

[0050] "Patient" or "subject" refers to a living organism suffering from or prone to a disease or condition that can be treated by administration of a pharmaceutical composition as provided herein. Non-limiting examples include humans, other mammals, bovines, rats, mice, dogs, monkeys, goat, sheep, cows, deer, and other non-mammalian animals. In aspects, a patient is human.

[0051] A "effective amount" is an amount sufficient for a compound to accomplish a stated purpose relative to the absence of the compound (e.g. achieve the effect for which it is administered, treat a disease, reduce enzyme activity, increase enzyme activity, reduce a signaling pathway, or reduce one or more symptoms of a disease or condition). An example of an "effective amount" is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a "therapeutically effective amount." A "reduction" of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). A "prophylactically effective amount" of a drug is an amount of a drug that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of an injury, disease, pathology or condition, or reducing the likelihood of the onset (or reoccurrence) of an injury, disease, pathology, or

condition, or their symptoms. The full prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a prophylactically effective amount may be administered in one or more administrations. An "activity decreasing amount," as used herein, refers to an amount of antagonist required to decrease the activity of an enzyme relative to the absence of the antagonist. A "function disrupting amount," as used herein, refers to the amount of antagonist required to disrupt the function of an enzyme or protein relative to the absence of the antagonist. The exact amounts will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, e.g., Lieberman, Pharmaceutical Dosage Forms (vols. 1-3, 1992); Lloyd, The Art, Science and Technology of Pharmaceutical Compounding (1999); Pickar, Dosage Calculations (1999); and Remington: The Science and Practice of Pharmacy, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins).

[0052] For any compound described herein, the therapeutically effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art.

[0053] As is well known in the art, therapeutically effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring compounds effectiveness and adjusting the dosage upwards or downwards, as described above. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

[0054] The term "therapeutically effective amount," as used herein, refers to that amount of the therapeutic agent sufficient to ameliorate the disorder, as described above. For example, for the given parameter, a therapeutically effective amount will show an increase or decrease of at least 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 80%, 90%, or at least 100%. Therapeutic efficacy can also be expressed as "-fold" increase or decrease. For example, a therapeutically effective amount can have at least a 1.2-fold, 1.5-fold, 2-fold, 5-fold, or more effect over a control.

[0055] Dosages may be varied depending upon the requirements of the patient and the compound being employed. The dose administered to a patient, in the context of the disclosure, should be sufficient to effect a beneficial therapeutic response in the patient over time. The size

of the dose also will be determined by the existence, nature, and extent of any adverse side-effects. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached. Dosage amounts and intervals can be adjusted individually to provide levels of the administered compound effective for the particular clinical indication being treated. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease state.

[0056] As used herein, the term "administering" means oral administration, administration as a suppository, topical contact, parenteral, intralesional, intrathecal, or intranasal administration, or the implantation of a slow-release device, *e.g.,* a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (e.g., buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, e.g., intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, etc. In aspects, the administering does not include administration of any active agent other than the recited active agent.

[0057] "Co-administer" it is meant that a composition described herein is administered at the same time, just prior to, or just after the administration of one or more additional therapies. The compounds provided herein can be administered alone or can be coadministered to the patient. Coadministration is meant to include simultaneous or sequential administration of the compounds individually or in combination (more than one compound). Thus, the preparations can also be combined, when desired, with other active substances (e.g. to reduce metabolic degradation). The compositions of the disclosure can be delivered transdermally, by a topical route, or formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols.

[0058] The terms “bind” and “bound” as used herein is used in accordance with its plain and ordinary meaning and refers to the association between atoms or molecules. The association can be direct or indirect. For example, bound atoms or molecules may be direct, e.g., by covalent bond or linker (e.g. a first linker or second linker), or indirect, e.g., by non-covalent bond (e.g. electrostatic interactions (e.g. ionic bond, hydrogen bond, halogen bond), van der Waals interactions (e.g. dipole-dipole, dipole-induced dipole, London dispersion), ring stacking (pi

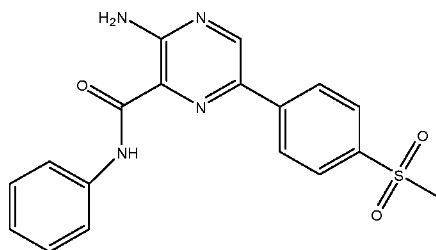
effects), hydrophobic interactions and the like).

[0059] The term “capable of binding” as used herein refers to a moiety (e.g. a compound as described herein) that is able to measurably bind to a target. In aspects, where a moiety is capable of binding a target, the moiety is capable of binding with a Kd of less than about 10 μM, 5 μM, 1 μM, 500 nM, 250 nM, 100 nM, 75 nM, 50 nM, 25 nM, 15 nM, 10 nM, 5 nM, 1 nM, or about 0.1 nM.

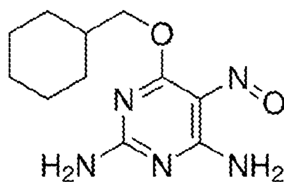
[0060] The term “pathway” or “signaling pathway” as used herein refers to a series of interactions between cellular and optionally extra-cellular components (e.g. proteins, nucleic acids, small molecules, ions, lipids) that conveys a change in one component to one or more other components, which in turn may convey a change to additional components, which is optionally propagated to other signaling pathway components.

[0061] “ATR kinase inhibitor,” as used herein refers to an inhibitor of ataxia telangiectasia and rad3-related (ATR) kinase, a DNA damage response kinase, with potential antineoplastic activity. ATR, a serine/threonine protein kinase, plays a key role in DNA repair, cell cycle progression, and survival, and is activated by DNA damage caused during DNA replication-associated stress. Exemplary ATR kinase inhibitors include berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, caffeine, wortmannin, or an analog of any one of the foregoing.

[0062] “VE-821” or “3-amino-6-(4-(methylsulfonyl)phenyl)-N-phenylpyrazine-2-carboxamide” refers to a compound having the structure:

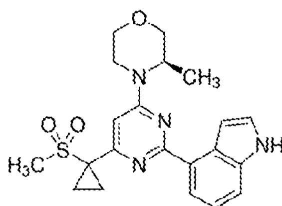


[0063] **“**NU6027” or “4-cyclohexylmethoxy-2,6-diamino-5-nitrosopyrimidine” refers to a compound having the structure:



[0064] “AZ20” or “4[4-[(3R)3-methylmorpholin-4-yl]-6-[1-(methylsulfonyl)cyclopropyl]-

pyrimidin-2-yl]-1H-indole" refers to a compound having the structure:



[0065] "Analog," or "analogue" is used in accordance with its plain ordinary meaning within Chemistry and Biology and refers to a chemical compound that is structurally similar to another compound (i.e., a so-called "reference" compound) but differs in composition, e.g., in the replacement of one atom by an atom of a different element, or in the presence of a particular functional group, or the replacement of one functional group by another functional group, or the absolute stereochemistry of one or more chiral centers of the reference compound. Accordingly, an analog is a compound that is similar or comparable in function and appearance but not in structure or origin to a reference compound.

[0066] "Contacting" is used in accordance with its plain ordinary meaning and refers to the process of allowing at least two distinct species (e.g. chemical compounds including biomolecules or cells) to become sufficiently proximal to react, interact or physically touch. It should be appreciated; however, the resulting reaction product can be produced directly from a reaction between the added reagents or from an intermediate from one or more of the added reagents that can be produced in the reaction mixture. In aspects contacting includes allowing a compound described herein to interact with a protein or enzyme that is involved in a signaling pathway.

[0067] As defined herein, the term "activation", "activate", "activating", "activator" and the like in reference to a protein-inhibitor interaction means positively affecting (e.g. increasing) the activity or function of the protein relative to the activity or function of the protein in the absence of the activator. In aspects activation means positively affecting (e.g. increasing) the concentration or levels of the protein relative to the concentration or level of the protein in the absence of the activator. The terms may reference activation, or activating, sensitizing, or up-regulating signal transduction or enzymatic activity or the amount of a protein decreased in a disease. Thus, activation may include, at least in part, partially or totally increasing stimulation, increasing or enabling activation, or activating, sensitizing, or up-regulating signal transduction or enzymatic activity or the amount of a protein associated with a disease (e.g., a protein which is decreased in a disease relative to a non-diseased control). Activation may include, at least in part, partially or totally increasing stimulation, increasing or enabling activation, or activating,

sensitizing, or up-regulating signal transduction or enzymatic activity or the amount of a protein

[0068] The terms “agonist,” “activator,” “upregulator,” etc. refer to a substance capable of detectably increasing the expression or activity of a given gene or protein. The agonist can increase expression or activity 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a control in the absence of the agonist. In certain instances, expression or activity is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or higher than the expression or activity in the absence of the agonist.

[0069] As defined herein, the term “inhibition”, “inhibit”, “inhibiting” and the like in reference to a cancer cell proliferation-inhibitor interaction means negatively affecting (e.g. decreasing) the growth, activity, or function of the cancer cell relative to the growth, activity, or function of the cancer cells in the absence of the inhibitor. In aspects inhibition means negatively affecting (e.g. decreasing) the concentration or levels of the cancer cells relative to the concentration or level of the cancer cells in the absence of the inhibitor. In aspects inhibition refers to reduction of a disease or symptoms of disease. In aspects, inhibition refers to a reduction in the activity of particular cancer cells. In aspects, inhibition refers to a reduction of activity or concentration of cancer cells resulting from a direct or indirect interaction between the inhibitor and the cancer cells.

[0070] The terms “inhibitor,” “repressor” or “antagonist” or “downregulator” interchangeably refer to a compound capable of detectably decreasing the growth, activity, or function of any given cancer cells. The antagonist can decrease growth, activity, or function of cancer cells by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a control in the absence of the antagonist. In aspects, growth, activity, or function of cancer cells is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or lower than the growth, activity, or function of cancer cells in the absence of the antagonist.

[0071] The term "expression" includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion. Expression can be detected using conventional techniques for detecting protein (e.g., ELISA, Western blotting, flow cytometry, immunofluorescence, immunohistochemistry, etc.).

[0072] The term “modulator” refers to a composition that increases or decreases the level of a target molecule or the function of a target molecule or the physical state of the target of the molecule relative to the absence of the modulator.

[0073] The term “modulate” is used in accordance with its plain ordinary meaning and refers

to the act of changing or varying one or more properties. "Modulation" refers to the process of changing or varying one or more properties. For example, as applied to the effects of a modulator on a target protein, to modulate means to change by increasing or decreasing a property or function of the target molecule or the amount of the target molecule.

[0074] The term "associated" or "associated with" in the context of a substance or substance activity or function associated with a disease (e.g. a protein associated disease, a cancer (e.g., cancer, inflammatory disease, autoimmune disease, or infectious disease)) means that the disease (e.g. cancer, inflammatory disease, autoimmune disease, or infectious disease) is caused by (in whole or in part), or a symptom of the disease is caused by (in whole or in part) the substance or substance activity or function. As used herein, what is described as being associated with a disease, if a causative agent, could be a target for treatment of the disease.

[0075] Assays and Methods for Treating Cancer

[0076] In embodiments, the disclosure provides methods for identifying a compound that inhibits cancer cell proliferation, the method comprising: (i) contacting the compound with cancer cells and salvage pathway condition-specific growth media and de novo pathway condition-specific growth media; wherein the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite; and (ii) identifying inhibition of cancer cell proliferation by the compound; thereby identifying the compound that inhibits cancer cell proliferation. In aspects, the salvage pathway is a pyrimidine nucleoside salvage pathway, the de novo pathway is a de novo nucleotide pathway, and the common metabolite is uridine monophosphate. In aspects, the compound that inhibits cancer cell proliferation is JNK-IN-8, CNX-774, motesanib, OSU-03012, TAK-632, or an analog of any of the foregoing. In aspects, the cancer cells are pancreatic cancer cells. In aspects, the cancer cells are pancreatic ductal adenocarcinoma cells. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation. In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma. In aspects, the method of treating cancer further comprises administering to the patient a therapeutically effective amount of an ATR kinase inhibitor.

[0077] In embodiments, the disclosure provides methods for identifying a compound that inhibits cancer cell proliferation, the method comprising: (i) contacting the compound with cancer cells and salvage pathway condition-specific growth media; wherein the salvage pathway, and its associated de novo pathway are convergent metabolic pathways producing a

common metabolite; and (ii) identifying inhibition of cancer cell proliferation by the compound; thereby identifying the compound that inhibits cancer cell proliferation. In aspects, the salvage pathway is a pyrimidine nucleoside salvage pathway, the de novo pathway is a de novo nucleotide pathway, and the common metabolite is uridine monophosphate. In aspects, the compound that inhibits cancer cell proliferation is JNK-IN-8, CNX-774, motesanib, or an analog of any of the foregoing. In aspects, the cancer cells are pancreatic cancer cells. In aspects, the cancer cells are pancreatic ductal adenocarcinoma cells. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation. In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma. In aspects, the method of treating cancer further comprises administering to the patient a therapeutically effective amount of an ATR kinase inhibitor.

[0078] In embodiments, the disclosure provides methods for identifying a compound that inhibits cancer cell proliferation, the method comprising: (i) contacting the compound with cancer cells and de novo pathway condition-specific growth media; wherein the de novo pathway, and its associated salvage pathway, are convergent metabolic pathways producing a common metabolite; wherein the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite; and (ii) identifying inhibition of cancer cell proliferation by the compound; thereby identifying the compound that inhibits cancer cell proliferation. In aspects, the salvage pathway is a pyrimidine nucleoside salvage pathway, the de novo pathway is a de novo nucleotide pathway, and the common metabolite is uridine monophosphate. In aspects, the compound that inhibits cancer cell proliferation is a dihydroorotate dehydrogenase inhibitor. In aspects, the compound that inhibits cancer cell proliferation is OSU-03012, TAK-632, or an analog of any of the foregoing. In aspects, the cancer cells are pancreatic cancer cells. In aspects, the cancer cells are pancreatic ductal adenocarcinoma cells. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation. In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma. In aspects, the method of treating cancer further comprises administering to the patient a therapeutically effective amount of an ATR kinase inhibitor.

[0079] In embodiments, the disclosure provides methods for identifying a compound that inhibits cancer cell proliferation, the method comprising: (i) contacting the compound with cancer cells and (a) salvage pathway condition-specific growth media and de novo pathway

condition-specific growth media and (b) salvage pathway condition-specific growth media; wherein the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite; and (ii) identifying inhibition of cancer cell proliferation by the compound in (a) and/or (b); thereby identifying the compound that inhibits cancer cell proliferation. In aspects, the salvage pathway is a pyrimidine nucleoside salvage pathway, the de novo pathway is a de novo nucleotide pathway, and the common metabolite is uridine monophosphate. In aspects, the cancer cells are pancreatic cancer cells. In aspects, the cancer cells are pancreatic ductal adenocarcinoma cells. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation in (a) and (b). In aspects, the compound that inhibits cancer cell proliferation in (a) and (b) is JNK-IN-8, CNX-774, motesanib, or an analog of any of the foregoing. In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation in (a) but not (b). In aspects, the compound that inhibits cancer cell proliferation is (a) but not (b) is OSU-03012, TAK-632, or an analog of any of the foregoing. In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma. In aspects, the method of treating cancer further comprises administering to the patient a therapeutically effective amount of an ATR kinase inhibitor.

[0080] In embodiments, the disclosure provides methods for identifying a compound that inhibits cancer cell proliferation, the method comprising: (i) contacting the compound with cancer cells and (a) salvage pathway condition-specific growth media and de novo pathway condition-specific growth media and (b) de novo pathway condition-specific growth media; wherein the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite; and (ii) identifying inhibition of cancer cell proliferation by the compound in (a) and/or (b); thereby identifying the compound that inhibits cancer cell proliferation. In aspects, the salvage pathway is a pyrimidine nucleoside salvage pathway, the de novo pathway is a de novo nucleotide pathway, and the common metabolite is uridine monophosphate. In aspects, the cancer cells are pancreatic cancer cells. In aspects, the cancer cells are pancreatic ductal adenocarcinoma cells. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation in (a) and (b). In aspects, the compound that inhibits cancer cell proliferation in (a) and (b) is OSU-03012, TAK-632, or

an analog of any of the foregoing. In aspects, the compound that inhibits cancer cell proliferation in (a) and (b) is a dihydroorotate dehydrogenase inhibitor. In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation in (a) but not (b). In aspects, the compound that inhibits cancer cell proliferation in (a) but not (b) is JNK-IN-8, CNX-774, motesanib, or an analog of any of the foregoing. In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma. In aspects, the method of treating cancer further comprises administering to the patient a therapeutically effective amount of an ATR kinase inhibitor.

[0081] In embodiments, the disclosure provides methods for identifying a compound that inhibits cancer cell proliferation, the method comprising: (i) contacting the compound with cancer cells and (a) salvage pathway condition-specific growth media and (b) de novo pathway condition-specific growth media; wherein the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite; and (ii) identifying inhibition of cancer cell proliferation by the compound in (a) and/or (b); thereby identifying the compound that inhibits cancer cell proliferation. In aspects, the salvage pathway is a pyrimidine nucleoside salvage pathway, the de novo pathway is a de novo nucleotide pathway, and the common metabolite is uridine monophosphate. In aspects, the cancer cells are pancreatic cancer cells. In aspects, the cancer cells are pancreatic ductal adenocarcinoma cells. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation in (a) and (b). In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation in (a) but not (b). In aspects, the compound that inhibits cancer cell proliferation in (a) but not (b) is JNK-IN-8, CNX-774, motesanib, or an analog of any of the foregoing. In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation in (b) but not (a). In aspects, the compound that inhibits cancer cell proliferation in (b) but not (a) is OSU-03012, TAK-632, or an analog of any of the foregoing. In aspects, the compound that inhibits cancer cell proliferation in (b) not (a) is a dihydroorotate dehydrogenase inhibitor. In aspects, the cancer is pancreatic cancer. In aspects,

the cancer is pancreatic ductal adenocarcinoma. In aspects, the method of treating cancer further comprises administering to the patient a therapeutically effective amount of an ATR kinase inhibitor.

[0082] In embodiments, the disclosure provides methods for identifying a compound that inhibits cancer cell proliferation, the method comprising: (i) contacting the compound with cancer cells and (a) salvage pathway condition-specific growth media and de novo pathway condition-specific growth media; (b) salvage pathway condition-specific growth media, and (c) de novo pathway condition-specific growth media; wherein the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite; and (ii) identifying inhibition of cancer cell proliferation by the compound in (a) and/or (b) and/or (c); thereby identifying the compound that inhibits cancer cell proliferation. In aspects, the salvage pathway is a pyrimidine nucleoside salvage pathway, the de novo pathway is a de novo nucleotide pathway, and the common metabolite is uridine monophosphate. In aspects, the cancer cells are pancreatic cancer cells. In aspects, the cancer cells are pancreatic ductal adenocarcinoma cells. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation in (a), (b), and (c). In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation in (a) and (b), but not (c). In aspects, the compound that inhibits cancer cell proliferation is JNK-IN-8, CNX-774, motesanib, or an analog of any of the foregoing. In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma. In aspects, the disclosure provides methods of treating cancer in patient in need thereof by administering to the patient a therapeutically effective amount of the compound that inhibits cancer cell proliferation in (a) and (c), but not (b). In aspects, the compound that inhibits cancer cell proliferation in (a) and (c) but not (b) is OSU-03012, TAK-632, or an analog of any of the foregoing. In aspects, the compound that inhibits cancer cell proliferation in (a) and (c) but not (b) is a dihydroorotate dehydrogenase inhibitor. In aspects, the cancer is pancreatic cancer. In aspects, the cancer is pancreatic ductal adenocarcinoma. In aspects, the method of treating cancer further comprises administering to the patient a therapeutically effective amount of an ATR kinase inhibitor.

[0083] Methods of Treating Cancer

[0084] In embodiments, the disclosure provides methods of treating cancer in a patient in need

thereof by administering to the patient a therapeutically effective amount of a compound that is a salvage pathway inhibitor but that is not a de novo pathway inhibitor. In embodiments, the disclosure provides methods of treating cancer in a patient in need thereof by administering to the patient a therapeutically effective amount of a compound that is a de novo pathway inhibitor but that is not a salvage pathway inhibitor. In aspects, the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite.

[0085] In embodiments, the disclosure provides methods of treating cancer in a patient in need thereof by administering to the patient a therapeutically effective amount of a compound that is a pyrimidine nucleoside salvage pathway inhibitor but that is not a de novo nucleotide pathway inhibitor. In aspects, the pyrimidine nucleoside salvage pathway and de novo nucleotide pathway are convergent metabolic pathways producing UMP as a common metabolite. In aspects, the compound that is a pyrimidine nucleoside salvage pathway inhibitor is JNK-IN-8, CNX-774, motesanib, or an analog of any of the foregoing. In aspects, the compound that is a pyrimidine nucleoside salvage pathway inhibitor but not a de novo nucleotide pathway inhibitor is JNK-IN-8. In aspects, the compound that is a pyrimidine nucleoside salvage pathway inhibitor but not a de novo nucleotide pathway inhibitor is CNX-774. In aspects, the compound that is a pyrimidine nucleoside salvage pathway inhibitor but not a de novo nucleotide pathway inhibitor is motesanib. In aspects, the methods further comprise administering to the patient a therapeutically effective amount of an ATR kinase inhibitor. In aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, caffeine, wortmannin, or an analog of any one of the foregoing.

[0086] In embodiments, the disclosure provides methods of treating pancreatic cancer in a patient in need thereof by administering to the patient a therapeutically effective amount of a compound that is a pyrimidine nucleoside salvage pathway inhibitor but that is not a de novo nucleotide pathway inhibitor. In aspects, the pyrimidine nucleoside salvage pathway and de novo nucleotide pathway are convergent metabolic pathways producing UMP as a common metabolite. In aspects, the compound that is a pyrimidine nucleoside salvage pathway inhibitor is JNK-IN-8, CNX-774, motesanib, or an analog of any of the foregoing. In aspects, the compound that is a pyrimidine nucleoside salvage pathway inhibitor but not a de novo nucleotide pathway inhibitor is JNK-IN-8. In aspects, the compound that is a pyrimidine nucleoside salvage pathway inhibitor but not a de novo nucleotide pathway inhibitor is CNX-774. In aspects, the compound that is a pyrimidine nucleoside salvage pathway inhibitor but not a de novo nucleotide pathway inhibitor is motesanib. In aspects, the methods further comprise administering to the patient a therapeutically effective amount of an ATR kinase inhibitor. In

aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, caffeine, wortmannin, or an analog of any one of the foregoing.

[0087] In embodiments, the disclosure provides methods of treating pancreatic ductal adenocarcinoma in a patient in need thereof by administering to the patient a therapeutically effective amount of a compound that is a pyrimidine nucleoside salvage pathway inhibitor but that is not a de novo nucleotide pathway inhibitor. In aspects, the pyrimidine nucleoside salvage pathway and de novo nucleotide pathway are convergent metabolic pathways producing UMP as a common metabolite. In aspects, the compound that is a pyrimidine nucleoside salvage pathway inhibitor is JNK-IN-8, CNX-774, motesanib, or an analog of any of the foregoing. In aspects, the compound that is a pyrimidine nucleoside salvage pathway inhibitor but not a de novo nucleotide pathway inhibitor is JNK-IN-8. In aspects, the compound that is a pyrimidine nucleoside salvage pathway inhibitor but not a de novo nucleotide pathway inhibitor is CNX-774. In aspects, the compound that is a pyrimidine nucleoside salvage pathway inhibitor but not a de novo nucleotide pathway inhibitor is motesanib. In aspects, the methods further comprise administering to the patient a therapeutically effective amount of an ATR kinase inhibitor. In aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, caffeine, wortmannin, or an analog of any one of the foregoing.

[0088] In embodiments, the disclosure provides methods of treating cancer in a patient in need thereof by administering to the patient a therapeutically effective amount of a compound that is a de novo nucleotide pathway inhibitor but that is not a pyrimidine nucleoside salvage pathway inhibitor. In aspects, the pyrimidine nucleoside salvage pathway and de novo nucleotide pathway are convergent metabolic pathways producing UMP as a common metabolite. In aspects, the compound that is a de novo nucleotide pathway inhibitor but not a pyrimidine nucleoside salvage pathway inhibitor is a dihydroorotate dehydrogenase inhibitor. In aspects, the compound that is a de novo nucleotide pathway inhibitor but not a pyrimidine nucleoside salvage pathway inhibitor is OSU-03012, TAK-632, or an analog of the foregoing. In aspects, the compound that is a de novo nucleotide pathway inhibitor but not a pyrimidine nucleoside salvage pathway inhibitor is OSU-03012. In aspects, the compound that is a de novo nucleotide pathway inhibitor but not a pyrimidine nucleoside salvage pathway inhibitor is TAK-632. In aspects, the methods further comprise administering to the patient a therapeutically effective amount of an ATR kinase inhibitor. In aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, caffeine, wortmannin, or an analog of any one of the foregoing.

[0089] In embodiments, the disclosure provides methods of treating pancreatic cancer in a patient in need thereof by administering to the patient a therapeutically effective amount of a compound that is a de novo nucleotide pathway inhibitor but that is not a pyrimidine nucleoside salvage pathway inhibitor. In aspects, the pyrimidine nucleoside salvage pathway and de novo nucleotide pathway are convergent metabolic pathways producing UMP as a common metabolite. In aspects, the compound that is a de novo nucleotide pathway inhibitor but not a pyrimidine nucleoside salvage pathway inhibitor is a dihydroorotate dehydrogenase inhibitor. In aspects, the compound that is a de novo nucleotide pathway inhibitor but not a pyrimidine nucleoside salvage pathway inhibitor is OSU-03012, TAK-632, or an analog of the foregoing. In aspects, the compound that is a de novo nucleotide pathway inhibitor but not a pyrimidine nucleoside salvage pathway inhibitor is OSU-03012. In aspects, the compound that is a de novo nucleotide pathway inhibitor but not a pyrimidine nucleoside salvage pathway inhibitor is TAK-632. In aspects, the methods further comprise administering to the patient a therapeutically effective amount of an ATR kinase inhibitor. In aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, caffeine, wortmannin, or an analog of any one of the foregoing.

[0090] In embodiments, the disclosure provides methods of treating pancreatic ductal adenocarcinoma in a patient in need thereof by administering to the patient a therapeutically effective amount of a compound that is a de novo nucleotide pathway inhibitor but that is not a pyrimidine nucleoside salvage pathway inhibitor. In aspects, the pyrimidine nucleoside salvage pathway and de novo nucleotide pathway are convergent metabolic pathways producing UMP as a common metabolite. In aspects, the compound that is a de novo nucleotide pathway inhibitor but not a pyrimidine nucleoside salvage pathway inhibitor is a dihydroorotate dehydrogenase inhibitor. In aspects, the compound that is a de novo nucleotide pathway inhibitor but not a pyrimidine nucleoside salvage pathway inhibitor is OSU-03012, TAK-632, or an analog of the foregoing. In aspects, the compound that is a de novo nucleotide pathway inhibitor but not a pyrimidine nucleoside salvage pathway inhibitor is OSU-03012. In aspects, the compound that is a de novo nucleotide pathway inhibitor but not a pyrimidine nucleoside salvage pathway inhibitor is TAK-632. In aspects, the methods further comprise administering to the patient a therapeutically effective amount of an ATR kinase inhibitor. In aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, caffeine, wortmannin, or an analog of any one of the foregoing.

[0091] In embodiments, the disclosure provides methods of treating cancer in a patient in need thereof by administering to the patient a therapeutically effective amount of dihydroorotate

dehydrogenase inhibitor. In aspects, the dihydroorotate dehydrogenase inhibitor is OSU-03012, TAK-632, or an analog of the foregoing. In aspects, the dihydroorotate dehydrogenase inhibitor is OSU-03012. In aspects, the dihydroorotate dehydrogenase inhibitor is TAK-632. In aspects, the methods further comprise administering to the patient a therapeutically effective amount of an ATR kinase inhibitor. In aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, caffeine, wortmannin, or an analog of any one of the foregoing.

[0092] In embodiments, the disclosure provides methods of treating pancreatic cancer in a patient in need thereof by administering to the patient a therapeutically effective amount of a dihydroorotate dehydrogenase inhibitor. In aspects, the dihydroorotate dehydrogenase inhibitor is OSU-03012, TAK-632, or an analog of the foregoing. In aspects, the dihydroorotate dehydrogenase inhibitor is OSU-03012. In aspects, the dihydroorotate dehydrogenase inhibitor is TAK-632. In aspects, the methods further comprise administering to the patient a therapeutically effective amount of an ATR kinase inhibitor. In aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, caffeine, wortmannin, or an analog of any one of the foregoing. In aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, or an analog of any one of the foregoing. In aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, or AZ20.

[0093] In embodiments, the disclosure provides methods of treating pancreatic ductal adenocarcinoma in a patient in need thereof by administering to the patient a therapeutically effective amount of a dihydroorotate dehydrogenase inhibitor. In aspects, the dihydroorotate dehydrogenase inhibitor is OSU-03012, TAK-632, or an analog of the foregoing. In aspects, the dihydroorotate dehydrogenase inhibitor is OSU-03012. In aspects, the dihydroorotate dehydrogenase inhibitor is TAK-632. In aspects, the methods further comprise administering to the patient a therapeutically effective amount of an ATR kinase inhibitor. In aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, caffeine, wortmannin, or an analog of any one of the foregoing. In aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, or an analog of any one of the foregoing. In aspects, the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, or AZ20.

[0094] The disclosure provides compositions comprising dihydroorotate dehydrogenase and a dihydroorotate dehydrogenase inhibitor. In aspects, the compositions are co-crystals. In aspects,

the dihydroorotate dehydrogenase and dihydroorotate dehydrogenase inhibitor are ionically bonded together. In aspects, the dihydroorotate dehydrogenase and dihydroorotate dehydrogenase inhibitor are bonded together via one or more hydrogen bonds. In aspects, the dihydroorotate dehydrogenase inhibitor is OSU-03012, TAK-632, or an analog of any of the foregoing. In aspects, the dihydroorotate dehydrogenase inhibitor is OSU-03012. In aspects, the dihydroorotate dehydrogenase inhibitor is TAK-632.

[0095] Any of the compounds described herein may be administered to a subject in a pharmaceutical composition further comprising a pharmaceutically acceptable excipient. The compositions are suitable for formulation and administration *in vitro* or *in vivo*. Suitable carriers and excipients and their formulations are known in the art and described, e.g., Remington: The Science and Practice of Pharmacy, 21st Ed, Lippicott Williams & Wilkins (2005).

[0096] "Pharmaceutically acceptable excipient" and "pharmaceutically acceptable carrier" refer to a substance that aids the administration of an active agent to and absorption by a subject and can be included in the compositions of the disclosure without causing a significant adverse toxicological effect on the patient. Non-limiting examples of pharmaceutically acceptable excipients include water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors, salt solutions (such as Ringer's solution), alcohols, oils, gelatins, carbohydrates such as lactose, amylose or starch, fatty acid esters, hydroxymethycellulose, polyvinyl pyrrolidine, and colors, and the like. Such preparations can be sterilized and, if desired, mixed with auxiliary agents such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, and/or aromatic substances and the like that do not deleteriously react with the compounds of the disclosure. One of skill in the art will recognize that other pharmaceutical excipients are useful.

[0097] Solutions of the pharmaceutical compositions can be prepared in water suitably mixed with a lipid or surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms. Solutions can be administered, e.g., parenterally, such as subcutaneously or intravenously (e.g., infusion or bolus).

[0098] Pharmaceutical compositions can be delivered via intranasal or inhalable solutions. The intranasal composition can be a spray, aerosol, or inhalant. The inhalable composition can be a spray, aerosol, or inhalant. Nasal solutions can be aqueous solutions designed to be

administered to the nasal passages in drops or sprays. Nasal solutions can be prepared so that they are similar in many respects to nasal secretions. Thus, the aqueous nasal solutions usually are isotonic and slightly buffered to maintain a pH of 5.5 to 6.5. In addition, antimicrobial preservatives, similar to those used in ophthalmic preparations and appropriate drug stabilizers, if required, may be included in the formulation. Various commercial nasal preparations are known in the art.

[0099] Oral formulations can include excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders. In aspects, oral pharmaceutical compositions will comprise an inert diluent or edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food. For oral therapeutic administration, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the compositions and preparations may, of course, be varied and may be between about 1 to about 75% of the weight of the unit. The amount of nucleic acids in such compositions is such that a suitable dosage can be obtained.

[0100] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered and the liquid diluent first rendered isotonic with sufficient saline or glucose. Aqueous solutions, in particular, sterile aqueous media, are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion.

[0101] Sterile injectable solutions can be prepared by incorporating the compounds in the required amount in the appropriate solvent followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium. Vacuum-drying and freeze-drying techniques, which yield a powder of the active ingredient plus any additional desired ingredients, can be used to prepare sterile powders for reconstitution of sterile injectable solutions. The preparation of more, or highly, concentrated solutions for direct injection is also contemplated. Dimethyl sulfoxide can be used as solvent for rapid penetration, delivering high concentrations of the active agents to a small area.

[0102] The dosage and frequency (single or multiple doses) of the compounds and compositions administered to a subject can vary depending upon a variety of factors, for example, whether the mammal suffers from another disease, and its route of administration; size, age, sex, health, body weight, body mass index, and diet of the recipient; nature and extent of symptoms of the disease being treated, kind of concurrent treatment, complications from the disease being treated or other health-related problems. Other therapeutic regimens or agents can be used in conjunction with the methods and recombinant proteins described herein. Adjustment and manipulation of established dosages (e.g., frequency and duration) are within the ability of the skilled artisan.

[0103] For any composition and compounds described herein, the effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of compounds that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art. As is known in the art, effective amounts of compounds for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring effectiveness and adjusting the dosage upwards or downwards, as described above. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

[0104] Dosages of the compounds may be varied depending upon the requirements of the patient. The dose administered to a patient should be sufficient to affect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects. Determination of the proper dosage for a particular situation is within the skill of the art. Dosage amounts and intervals can be adjusted individually to provide levels of the compounds effective for the particular clinical indication being treated. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease state.

[0105] Utilizing the teachings provided herein, an effective prophylactic or therapeutic treatment regimen can be planned that does not cause substantial toxicity and yet is effective to treat the clinical disease or symptoms demonstrated by the particular patient. This planning should involve the careful choice of recombinant proteins by considering factors such as compound potency, relative bioavailability, patient body weight, presence and severity of adverse side effects.

[0106] In embodiments, the compounds are administered to a patient at an amount of about 0.001 mg/kg to about 500 mg/kg. In aspects, the compounds are administered to a patient in an amount of about 0.01 mg/kg, 0.1 mg/kg, 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, 40 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg, 200 mg/kg, or 300 mg/kg. It is understood that where the amount is referred to as "mg/kg," the amount is milligram per kilogram body weight of the subject being administered with the recombinant proteins. In aspects, the compounds are administered to a patient in an amount from about 0.01 mg to about 500 mg per day, as a single dose, or in a dose administered two or three times per day.

[0107] Embodiments

[0108] Embodiment P1. A method for identifying a compound that inhibits cancer cell proliferation, the method comprising: (i) contacting the compound with (a) cancer cells and salvage pathway condition-specific growth media and de novo pathway condition-specific growth media; and/or (b) cancer cells and salvage pathway condition-specific growth media; and/or (c) cancer cells and de novo pathway condition-specific growth media; wherein the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite; and (ii) identifying inhibition of cancer cell proliferation by the compound; thereby identifying the compound that inhibits cancer cell proliferation.

[0109] Embodiment P2. The method of Embodiment P1, wherein (b) does not comprise de novo pathway condition-specific growth media; and wherein (c) does not comprise salvage pathway condition-specific growth media.

[0110] Embodiment P3. The method of Embodiment P1 or P2, wherein: (a) comprises cancer cells and pyrimidine nucleoside salvage pathway condition-specific growth media and de novo nucleotide pathway condition-specific growth media; (b) comprises cancer cells and pyrimidine nucleoside salvage pathway condition-specific growth media; and (c) comprises cancer cells and de novo nucleotide pathway condition-specific growth media.

[0111] Embodiment P4. The method of any one of Embodiments P1 to P3, wherein the method comprises contacting the compound with (a) and (b).

[0112] Embodiment P5. The method of any one of Embodiments P1 to P3, wherein the method comprises contacting the compound with (a) and (c).

[0113] Embodiment P6. The method of any one of Embodiments P1 to P3, wherein the method comprises contacting the compound with (b) and (c).

[0114] Embodiment P7. The method of any one of Embodiments P1 to P3, wherein the method comprises contacting the compound with (a), (b), and (c).

[0115] Embodiment P8. A method of treating cancer in a patient in need thereof, the method comprising: (i) identifying the compound that inhibits cancer cell proliferation according to any one of Embodiments P1 to P7; (ii) selecting the compound that inhibits caner cell proliferation in (b) or (c); and (iii) administering to the patient a therapeutically effective amount of the compound that inhibits caner cell proliferation in (b) or (c).

[0116] Embodiment P9. The method of Embodiment P8, wherein the compound that inhibits caner cell proliferation in (b) does not inhibit cancer cell proliferation in (c).

[0117] Embodiment P10. The method of Embodiment P8 or P9, comprising selecting the compound that inhibits caner cell proliferation in (b); and administering to the patient a therapeutically effective amount of the compound that inhibits caner cell proliferation in (b).

[0118] Embodiment P11. The method of any one of Embodiments P8 to P10, wherein the compound that inhibits caner cell proliferation in (b) is JNK-IN-8, CNX-774, or motesanib.

[0119] Embodiment P12. The method of Embodiment P8, wherein the compound that inhibits caner cell proliferation in (c) does not inhibit cancer cell proliferation in (b).

[0120] Embodiment P13. The method of Embodiment P8 or P12, comprising selecting the compound that inhibits caner cell proliferation in (c); and administering to the patient a therapeutically effective amount of the compound that inhibits caner cell proliferation in (c).

[0121] Embodiment P14. The method of Embodiment P8, P12, or P13, wherein compound that inhibits caner cell proliferation in (c) is OSU-03012 or TAK-632.

[0122] Embodiment P15. The method of any one of Embodiments P1 to P13, wherein the cancer is pancreatic cancer.

[0123] Embodiment P16. The method of Embodiment P15, wherein the pancreatic cancer is pancreatic ductal adenocarcinoma.

[0124] Embodiment P17. A method of treating pancreatic cancer in a patient in need thereof, the method comprising administering a therapeutically effective amount of a de novo nucleotide pathway inhibitor.

[0125] Embodiment P18. The method of Embodiment P17, wherein the de novo nucleotide pathway inhibitor is a dihydroorotate dehydrogenase inhibitor.

[0126] Embodiment P19. The method of Embodiment P17 or P18, wherein the de novo

nucleotide pathway inhibitor is OSU-03012 or TAK-632.

[0127] Embodiment P20. The method of any one of Embodiments P17 to P19, wherein the de novo nucleotide pathway inhibitor is not a pyrimidine nucleoside salvage pathway inhibitor.

[0128] Embodiment P21. A method of treating pancreatic cancer in a patient in need thereof, the method comprising administering a therapeutically effective amount of a pyrimidine nucleoside salvage pathway inhibitor.

[0129] Embodiment P22. The method of Embodiment P21, wherein the pyrimidine nucleoside salvage pathway inhibitor is JNK-IN-8, CNX-774, or motesanib.

[0130] Embodiment P23. The method of Embodiment P21 or P22, wherein the pyrimidine nucleoside salvage pathway inhibitor is not a de novo nucleotide pathway inhibitor.

[0131] Embodiment P24. The method of any one of Embodiments P17 to P23, wherein the pancreatic cancer is pancreatic ductal adenocarcinoma.

[0132] Embodiment P25. The method of any one of Embodiments P8 to P24, further comprising administering to the patient a therapeutically effective amount of an ATR kinase inhibitor.

[0133] Embodiment P26. The method of Embodiment P25, wherein the ATR kinase inhibitor is berzosertib, VE-821, ceralasertib, schisandrin B, NU6027, dactolisib, AZ20, caffeine, wortmannin, or an analog of any one of the foregoing.

[0134] Embodiment P27. The method of any one of Embodiments P8 to P24, further comprising administering to the patient a therapeutically effective amount of an anti-cancer agent.

[0135] Embodiment P28. A composition comprising dihydroorotate dehydrogenase and TAK-632.

[0136] Embodiment P29. The composition of Embodiment P28, wherein TAK-632 and dihydroorotate dehydrogenase are bonded together via a hydrogen bond.

[0137] Embodiment P30. A composition comprising dihydroorotate dehydrogenase and OSU-03012.

[0138] Embodiment P31. The composition of Embodiment P30, wherein OSU-03012 and dihydroorotate dehydrogenase are bonded together via a hydrogen bond.

EXAMPLES

[0139] The following examples are for purposes of illustration and are not intended to limit the spirit or scope of the disclosure or claims.

[0140] A cell-based metabolic modifier screening platform for the discovery of modulators of convergent pyrimidine nucleotide biosynthetic pathways was designed and implemented. In screening a library of protein kinase inhibitors, multiple compounds were shown to possess previously uncharacterized nucleotide metabolism-modifying activity. The JNK inhibitor JNK-IN-8 was found to be a potent inhibitor of nucleoside transport, a property which was confirmed using nucleoside-analog Positron Emission Tomography (PET) imaging in mice. The PDK1 inhibitor OSU-03012 (also known as AR-12) and the RAF inhibitor TAK-632 were shown to inhibit the therapeutically relevant enzyme dihydroorotate dehydrogenase (DHODH) and their affinities were unambiguously confirmed through *in vitro* assays and co-crystallization with human DHODH.

[0141] In this study, the inventors showed that the pyrimidine NSP and DNP are interchangeable in their ability to sustain cancer cell proliferation and that a synthetic lethal phenotype can be achieved through their simultaneous inhibition. With this knowledge, a metabolic modifier screen was constructed for identifying selective modulators of NSP and DNP pathways. In screening a library of protein kinase inhibitors, it was discovered that the c-Jun N-terminal kinase (JNK) inhibitor JNK-IN-8 was a potent inhibitor of nucleoside transporters vital for NSP function, and that the 3-phosphoinositide-dependent protein kinase 1 (PDK1) inhibitor OSU-03012 (also known as AR-12) and the pan-RAF inhibitor TAK-632 both binded and inhibited the pyrimidine DNP enzyme DHODH.

[0142] Design of a differential metabolic modifier screen for identification of novel modulators of pyrimidine nucleotide metabolism

[0143] While redundant routes for UMP biosynthesis can complicate targeting, impaired proliferation resulting from simultaneous restriction of both de novo (DNP) and salvage (NSP) pathways can be used to identify selective modifiers of either NSP or DNP pathway activity (FIG. 5A). A differential metabolic modifier screen was developed for the discovery of small molecule modulators of UMP production by leveraging this biosynthetic redundancy. This cell-based screening platform concurrently tests the effects of small molecule compounds on the proliferation of cells in baseline (both NSP and DNP active), NSP-only, and DNP-only conditions (FIG. 1A). Molecules which inhibited proliferation in baseline conditions were classified as non-specific inhibitors. Those which inhibited proliferation in NSP-only conditions

are NSP inhibitors, while those that inhibited growth in DNP-only conditions are DNP inhibitors. The screen design was validated using the known DHODH inhibitor NITD-982 (Wang et al., 2011) and the FDA-approved nucleotide transport inhibitor dipyridamole (DPA), using CellTiter-Glo (CTG) to evaluate proliferation impairment (FIG. 5B).

[0144] Cancer cell lines exhibited varying degrees of sensitivity to NITD-982 (as determined by doubling-time-normalized proliferation inhibition) and were all rescued by uridine (rU) supplementation (FIG. 6)(Hafner et al., 2016). MIAPACA2 PDAC cells were utilized in the screen due to their ability to maintain baseline proliferation levels in NSP-only or DNP-only conditions, while also exhibiting a significant decrease in proliferation upon simultaneous NSP and DNP inhibition (FIG. 7A).

[0145] A library of 430 protein kinase inhibitors was chosen for evaluation, the rationale being twofold. First, it was hypothesized that the synthetic lethality screen may identify compounds that indirectly targeted pyrimidine metabolism by inhibiting regulatory signal transduction pathways. Second, because the majority of kinase inhibitors are ATP-mimetics, and given their resemblance to nucleotides, it was predicted that protein kinase inhibitors may posses additional, non-canonical targets within nucleotide metabolism. Consistently, several protein kinase inhibitors, specifically those exhibiting similarities with the phenylamino pyrimidine (PAP) imatinib scaffold, and a subset of p38 MAPK inhibitors, exhibited activity against nucleoside transporters (Damaraju et al., 2016; Huang et al., 2002). The library was screened at 7-point dose response in duplicate and composite NSP and DNP pathway selectivity scores were calculated for each compound as the sum of condition-specific anti-proliferative effects across the dose range (FIG. 7B). Assay quality was monitored using the Z-factor metric (FIG. 7C)(Ji-Hu Zhang, Kevin R. Oldenburg, 1999).

[0146] The JNK inhibitor JNK-IN-8, the BTK inhibitor CNX-774, and the VEGFR inhibitor motesanib were active in the NSP-only condition (FIG. 1B). The selectivity of these hits was unique among inhibitors of JNK (FIG. 1C), BTK, and VEGFR (FIGS. 7D-7E), indicating this phenotype likely did not result from on-target effects.

[0147] The PDK1 inhibitor OSU-03012 (also known as AR-12) and the pan-RAF inhibitor TAK-632 were identified as eliciting potent and selective inhibition of proliferation in the DNP-only condition (FIG. 1D)(Zhu et al., 2004; Okaniwa et al., 2013). Among the four PDK1 inhibitors and 14 RAF inhibitors tested, OSU-03012 and TAK-632 were unique in their ability to selectively inhibit the DNP, suggesting that their ability to inhibit the pyrimidine DNP was not the consequence of on-target effects (FIGS. 1E-1F). Hit selectivity was confirmed using an

orthogonal long-term proliferation inhibition assay (FIG. 7F).

[0148] JNK-IN-8 inhibits nucleoside uptake *in vitro* and *in vivo*

[0149] While three protein kinase inhibitors were identified as selective inhibitors of the pyrimidine NSP, JNK-IN-8 was exceptionally potent with IC_{50} values in the low nanomolar range. We reasoned that the activity of JNK-IN-8 could arise from either the inhibition of nucleoside shuttling across the plasma membrane, achieved by concentrative (CNT) or equilibrative (ENT) nucleoside transporters, or of pyrimidine nucleoside phosphorylation by UCKs. To determine the level at which JNK-IN-8 is active, the uptake of tritiated rU and deoxycytidine (dC) was quantified. These nucleosides rely upon the same equilibrative transporters (ENT1/2) to enter the cell but require different kinases (UCKs in the case of rU and deoxycytidine kinase - dCK- in the case of dC (Le et al., 2017)) for conversion into their respective monophosphate forms and resultant intracellular accumulation (FIG. 2A). The assay revealed that JNK-IN-8 inhibited the uptake of both rU and dC with similar potency (33 nM and 31 nM, respectively), indicating that the compound was inhibiting nucleoside transport (FIG. 2B). Additionally, JNK-IN-8 treatment rescued JURKAT cells from the anti-proliferative effects of gemcitabine, a dCK-dependent nucleoside analog prodrug which relies upon nucleoside transporters to enter the cell, with an EC_{50} of 15.2 nM. Similar rescue was observed with CNX-774, motesanib and DPA treatment (FIGS. 8A-8B).

[0150] After demonstrating the ability of JNK-IN-8 to inhibit the pyrimidine NSP *in vitro*, we assessed its ability to block accumulation of a dCK-specific nucleoside analog PET probe, ^{18}F-clofarabine ([^{18}F]CFA), in CCRF-CEM xenograft tumors formed in NSG mice (Kim et al., 2016). Following treatment, PET imaging revealed that tumor [^{18}F]CFA PET uptake was decreased in JNK-IN-8-treated mice, relative to vehicle-treated controls (FIGS. 2C-2D).

[0151] OSU-03012 and TAK-632 target *de novo* UMP biosynthesis and activate the DNA replication stress response pathway

[0152] Two kinase inhibitors, TAK-632 and OSU-03012, were identified as potent and selective inhibitors of the DNP (FIG. 8C). We reasoned that these compounds could target either CAD, DHODH, or UMPS enzymes in *de novo* pyrimidine biosynthesis (FIG. 3A). Both compounds induced S-phase arrest in MIAPACA2 cells, a phenotype associated with insufficient dNTP biosynthesis to sustain DNA replication and activation of intra-S phase signaling checkpoints. This effect was rescued by orotate (the product of DHODH) supplementation and could be completely reversed by rU supplementation (FIGS. 3B-3C). DHODH inhibition emerged as a likely mechanism, as it catalyzes one of three committed steps

within the DNP and is a druggable protein (Madak et al., 2019). Additionally, both OSU-03012 and TAK-632 posses fluorine substituents which have been shown to stabilize bioactive conformations of DHODH inhibitors (Bonomo et al., 2013; Baumgartner et al., 2006). In an *in vitro* colorimetric recombinant human DHODH activity assay, TAK-632 and OSU-03012 both inhibited DHODH activity in a dose-dependent manner (FIG. 3D) (Baumgartner et al., 2006). Importantly, the response to TAK-632 or OSU-03012 correlated with the response to a known DHODH inhibitor in this cell line panel (FIG. 3E).

[0153] OSU-03012 was recently reported to synergize with replication stress response kinase inhibitors in RSK-subtype mutant KRAS cancer models (Yuan et al., 2018). However, after confirming that OSU-03012 binds DHODH, we hypothesized that the observed synergy resulted from DHODH inhibition rather than PDK1 inhibition. Immunoblot analysis of S6K and S6 phosphorylation, PDK1 downstream targets, confirmed that GSK-2334470, a known PDK1 inhibitor, potently blocked PDK1 while OSU-03012 triggered S345 CHEK1 phosphorylation, a replication stress biomarker, only in the absence of rU (FIG. 3F). Similarly, TAK-632 only triggered CHEK1 phosphorylation in the absence of rU (FIG. 3G). MIAPACA2 cells were treated with M6620, an inhibitor of the proximal replication stress response kinase ATR, and either OSU-03012 or GSK-2334470 for 72 hours. A synergistic increase in cell death was observed when OSU-03012 and an ATR inhibitor were combined, whereas the combination of GSK-2334470 and M6620 demonstrated only a nominal increase in cell death as determined by AnnexinV/PI flow cytometry (FIGS. 3H and 10). Taken together, these data indicate that replication stress triggered by OSU-03012 is the consequence of DHODH inhibition rather than inhibition of its canonical target.

[0154] Co-crystal structures of OSU-03012 and TAK-632 in complex with human DHODH

[0155] To determine the molecular interactions between the protein and its putative inhibitors, complete DHODH co-crystallization data sets were obtained and processed to 1.4 Å and 2.7 Å for OSU-03012 and TAK-632, respectively (FIG. 9A). Both compounds bind in a hydrophobic channel composed by two N-domain α-helices through which ubiquinone travels, consistent with previously identified DHODH inhibitors (Baumgartner et al., 2006). A long-range hydrogen bond between Arg 69 and OSU-03012 helps orient the molecule to the hydrophobic pocket where the phenanthrene moiety inserts, while the remainder of the molecule lays on the outer surface of DHODH, blocking the hydrophobic channel (FIGS. 4A and 9B). Three hydrogen bonds stabilize TAK-632 in the same hydrophobic pocket: two with Tyr 37 and Leu 66 help stabilize the inhibitor at the opening of the channel, while a third with Gln 46 helps pull

the inhibitor deep into the pocket (FIGS. 4B and 9C).

[0156] Discussion

[0157] The screening strategy expands on previous "nutrient-sensitized" genetic and small molecule screens, the general design of which can be applied to any number of metabolic systems, so long as the system produces a critical metabolite through parallel and redundant biosynthetic pathways (Arroyo et al., 2016; Gohil et al., 2010). UMP biosynthesis (i.e. pyrimidine metabolism) was identified as an amenable extension for this type of screen, as it consists of convergent (de novo and salvage) pathways, and UMP depletion triggers a quantifiable change in cellular proliferation phenotype.

[0158] JNK-IN-8, developed as an irreversible inhibitor of c-Jun N-terminal kinases 1, 2, and 3 with low-nanomolar affinity, was the most potent of three NSP inhibitors identified (Zhang et al., 2012). The data show that it is additionally a potent inhibitor of nucleoside transport, both *in vitro* and *in vivo*. Thus, JNK-IN-8 should not be used in conjunction with compounds, including cancer-treating antimetabolites, which rely upon nucleoside transport for their research or therapeutic purpose. Furthermore, JNK-IN-8 should not be used in research settings wherein upregulation of the pyrimidine DNP may confound experimental results.

[0159] The structurally and functionally unrelated OSU-03012 and TAK-632 were identified as inhibitors of the pyrimidine DNP. This work is the first to unambiguously confirm the affinity of OSU-03012 and TAK-632 for DHODH through crystallography studies. Notably, the studies herein show that OSU-03012 and TAK-632 bind in the same hydrophobic tunnel of DHODH as known inhibitors brequinar and teriflunomide (the active metabolite of leflunomide). This indicates that these two protein kinase inhibitors compete with ubiquinone, a redox partner of DHODH which traverses the hydrophobic tunnel to regenerate FMN from $FMNH_2$. By competitively inhibiting the binding of ubiquinone, these compounds prevent DHODH from completing its redox cycle effectively abrogating its activity.

[0160] OSU-03012 has orphan drug designation in the European Union for treatment of tularaemia and cryptococcosis. We hypothesize that its effectiveness in these indications stems from its ability to inhibit DHODH, rather than from 'on-target' effects against PDK1. Indeed, DHODH inhibitors have demonstrated efficacy against viruses such as dengue virus and respiratory syncytial virus (Bonavia et al., 2011; Yang et al., 2018; Wang et al., 2011). In anticancer settings, OSU-03012 was recently demonstrated to synergize with CHK1 inhibitors in KRAS-mutant cancers (Yuan et al., 2018), which was initially attributed to its ability to inhibit PDK1. However, our data show that GSK-2334470, a PDK1 inhibitor more potent than OSU-

03012, displayed little synergy with ATR inhibition. In light of our crystallographic data, we conclude that the synergy observed between OSU-03012 and ATR inhibition is likely a result of the DHODH-inhibitory ability of the former. Taken together, our data indicate that DHODH inhibitors have utility in oncology, particularly if used in conjunction with ATR inhibitors or other DNA-damage response/replication stress response pathway inhibitors (Le et al., 2017).

[0161] In summary, the inventors designed and applied a metabolic modifier screen which identified multiple protein kinase inhibitors as having non-canonical targets within pyrimidine metabolism. Similarly constructed phenotypic screens designed against other metabolic networks containing convergent nodes may find use in drug discovery campaigns or in repurposing screens using existing compounds.

[0162] Experimental Model and Subject Details

[0163] Cell Culture. All cell cultures were between passages 3 and 20 and maintained in antibiotic free DMEM or RPMI +10% dialyzed FBS, at 37ºC in 5% CO_2. Cell cultures were monitored for mycoplasma contamination using the PCR-based Venor Mycoplasma kit. PDAC cell lines were acquired either from a commercial vendor (ATCC, DSMZ) or from collaborators. Cell line identity was independently authenticated by PCR.

[0164] Drugs. Drug stocks were prepared in DMSO or H_2O and diluted fresh in cell culture media for treatments. NITD-982 was synthesized as previously described by Bonavia et al, Proc Natl Acad Sci USA, 108(17), 6739-6744.

[0165] In Vivo Mouse Studies. All animal studies were approved by the UCLA Animal Research Committee (ARC). 4-6 week-old female NSG mice, obtained from UCLA Radiation Oncology, were injected subcutaneously on bilateral flanks with 0.5×10^6 CCRF-CEM cells suspended 1:1 in PBS:matrigel. 20 d post-inoculation treatment was initiated. JNK-IN-8 was suspended in 2% ethanol and 5% Tween-80 in PBS and administered by intraperitoneal (i.p.) injection at 25 mg/kg.

[0166] Method Details

[0167] Protein Kinase Inhibitor Phenotypic Screen. A library of 430 protein kinase inhibitors was arrayed in polypropylene 384-well plates at 200x concentrations covering a 7-point concentration range (corresponding to 1x concentrations: 5μM, 1.65μM, 550nM, 185nM, 61.5nM, 20.6nM, 6.85nM). 25μl per well of condition-specific growth media (DNP+NSP: media +10 μM rU; DNP: media alone; NSP: media +10 μM rU + 1 μM NITD-982) was plated in opaque-white 384-well plates using a BioTek multidrop liquid handler. Protein kinase

inhibitors were added by 250 nL pin-tool transfer (BioMek FX, Beckman-Coulter) and inhibitor/media mixtures were incubated at room temperature for 30 m. 25 µL of a 40,000 cells/mL MIAPACA2 suspension (for 1000 cells/well) was subsequently added to each well. After 72 h, 50 µL of CellTiter-Glo reagent diluted 1:4 in dH_2O was added to each well and luminescence was measured using a Wallac plate reader (Perkin Elmer). Each condition was assayed in duplicate (n=2) and % proliferation values were calculated by normalizing experimental wells to plate negative controls and averaging replicate values. Composite pathway selectivity synergy scores for each test compound were defined as the sum of the excess over additivity (% proliferation inhibition observed - % proliferation inhibition expected) between individual protein kinase inhibitor concentrations across the 7-point concentration range. Z factor scores for individual assay plates were calculated using eight positive and eight negative control wells on each plate as previously described (Ji-Hu Zhang, 1999). All plates gave a Z factor > 0.5 (FIG. 7C).

[0168] ^{3}H-rU and ^{3}H-dC Uptake Assays. Radioactive probe uptake assays were conducted as previously described (Campbell et al., 2011). Briefly, 5×10^5 CCRF-CEM cells were resuspended in 1 mL of media per well in 12-well plates. After 1 h, cells were incubated with the indicated amounts of tritiated probe ± JNK-IN-8 for an additional hour. Cells were then harvested and washed twice with ice-cold PBS. Radioactivity was measured using a beta-counter (Perkin-Elmer).

[0169] Positron Emission Tomography (PET) imaging. MicroPET/CT experiments were conducted as previously described (Shu et al., 2010). Briefly, prewarmed and anesthetized NSG mice were injected with [^{18}F]CFA, and PET and CT images were acquired using the G8 PET/CT scanner (Sofie Biosciences) 4 h after i.p. administration of JNK-IN-8 or its vehicle and 3 h after the injection of 740 kBq of [^{18}F]CFA.

[0170] Radiochemical Synthesis of [^{18}F]-Labeled Probes. The syntheses of [^{18}F]CFA was performed as previously described by Shu et al., *J Nucl Med*, *51*(7), 1092-1098 (2010).

[0171] Flow cytometry. All flow cytometry data were acquired on five-laser BD LSRII, and analyzed using FlowJo software (Tree Star).

[0172] AnnexinV/PI: Treated PDAC cells were washed with PBS and incubated with AnnexinV and propidium iodide diluted in 1x Annexin binding buffer. 20,000 events were collected per sample.

[0173] Propidium Iodide Cell Cycle Analysis: Treated PDAC cells were washed with PBS and suspended in propidium iodide cell cycle staining solution (100 µg/ml propidium iodide; 20

μg/ml Ribonuclease A). 10,000 events were collected per sample.

[0174] Viability Analysis. For CellTiter-Glo analysis cells were plated at $1x10^3$ cells / well in at 50 μl / well in white opaque 384-well plates and treated as described. Following incubation 50 μl of CellTiter-Glo reagent (Diluted 1:5 in dH_2O) was added to each well, plates incubated at room temperature for 5 m and luminescence was measured using a BioTek microplate luminescence reader. Proliferation rate normalized growth inhibition was calculated using the previously described GR metric (Hafner et al., 2016).

[0175] For crystal violet staining, PDAC cells were plated in 6-well cell culture plates at $1x10^4$ cells/well and treated as described. Following treatment cells were fixed by incubating in 4% PFA in PBS for 15 m at room temperature. Plates were subsequently washed with PBS and stained with 0.1% crystal violet in H_2O for 15 m at room temperature.

[0176] Gene cloning, protein expression, and purification of DHODH in E.coli cells. Primers were ordered to add NdeI (described in Abt et al, Cell Chemical Biology, 27(2):197-205 (2019) and US Application No. 62/848,728 filed May 16, 2019) upstream of residue 29 (after the mitochondrial membrane associated loop) and BamHI (described in Abt et al, Cell Chemical Biology, 27(2):197-205 (2019) and US Application No. 62/848,728 filed May 16, 2019) after the stop codon to insert into N-terminal His-Sumo pET 14b vector. This clone, His-Sumo-DHODH 29-395 (subsequently referred to as DHODH) was successfully inserted into the vector in XL1-blue cells for vector propagation.

[0177] The vector was transformed into C41(DE3) cells for productions. Cells were grown at 37°C in 2xYT medium supplemented with 100 μg/mL ampicillin (Amp), treated with 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at an OD600 nm of 0.6-0.8, and then cultured for an additional 18h at 18°C. Cells were harvested by centrifugation, washed with 200 mM NaCl and 25 mM Tris pH 7.5, and pelleted at 5000 rpm for 20 minutes before storage at -20°C. 6.7g/L of cell pellet was obtained.

[0178] DHODH was purified according to known purification conditions (Baumgartner et al., 2006). Cell pellet was resuspended in lysis buffer (50 mM Tris pH 7.5; 600 mM NaCl; 0.33% w/v Thesit; 10% Glycerol; 1 mM PMSF) and lysed by sonication on ice. Lysed cells were centrifuged at 58,500 RCF for 45 minutes at 4°C, and the supernatant was filtered through a 0.45 μM filter and loaded onto a 5-mL His-Trap column pre-equilibrated with buffer A (50 mM Tris pH 7.4; 600 mM NaCl; 0.05% w/v Thesit; 10% Glycerol). The column was washed with buffer A for 70 mL, buffer A with 25 mM imidazole for 50 mL, and buffer A with 50 mM imidazole for 50 mL. The protein was eluted with buffer A with 250 mM imidazole. The eluted fraction

was diluted 1:1 with Buffer A. Sumo protease was added and the protein was dialyzed overnight at 4°C against 1L of Buffer A. The dialyzed protein was loaded back onto the His-Trap column equilibrated with buffer A. The cut-DHODH was eluted with buffer A with 50 mM imidazole. The purified protein was concentrated to 5 mL and injected onto S-200 gel filtration column (GE Healthcare) equilibrated with: 50 mM HEPES pH 7.7, 400 mM NaCl, 10% Glycerol, 1mM EDTA, 0.05 % w/v Thesit. Eluted fractions consistent with monomer size were collected, concentrated, flash frozen, and stored at -80°C.

[0179] Recombinant DHODH Enzyme Assay. Evaluation of DHODH inhibition was performed as previously described (Baumgartner et al., 2006). The standard assay mixture contained 50 µM decyclo-ubiquinone, 100 µM dihydroorotate, and 60 µM 2,6-dichloroindophenol (DCIP). The amount of DHODH was 337.4 ng/mL. Measurements were conducted in 50 mM TrisHCl, 150 mM KCl, 0.1% Triton X-100, pH 8.0, at 30 °C in a final volume of 1 mL. The components were mixed, and the reaction was started by adding dihydroorotate. The reaction was followed spectrophotometrically by measuring the decrease in absorption at 600 nm for 2.5 min at 30 second intervals. The assay was linear in time and enzyme concentration. Inhibitory studies were conducted in a standard assay with additional variable amounts of inhibitor.

[0180] Crystallization of DHODH with OSU-03012 and TAK-632 Compounds. For co-crystallization of DHODH and OSU-03012, crystals were obtained using the same conditions reported in previously published DHODH structures (Lewis et al., 2016; Baumgartner et al., 2006; Das et al., 2013; Davies et al., 2009; Erra et al., 2011; Hurt, Sutton, & Clardy, 2006; Ladds et al., 2018; Liu, Neidhardt, Grossman, Ocain, & Clardy, 2000; McLean et al., 2010; Sainas et al., 2018; Walse et al., 2008), namely 1.6 – 2.6 M ammonium sulfate and 5-30% glycerol in the well in pH 4.5, with 20 mg/mL DHODH with 2 mM dihydroorotate (DHO), 20.8 mM dodecyldimethyl-N-amineoxide (DDAO), and 400 µM inhibitor. Protein was mixed 1:1 with mother liquor and hanging drops were used at room temperature. Crystals appeared after 48 hours and reached maximal size within one week. Molecular replacement used 4OQV (Deng et al., 2014) as the starting model. Interestingly, DHODH-TAK-632 crystals grown in similar conditions to those used for the DHODH-OSU-03012 complex did not show TAK-632 density. As a result, novel DHODH crystallization conditions were identified using commercial screens. For co-crystallization of DHODH with TAK-632, crystals were obtained in conditions of 1.4-1.6 M sodium phosphate, pH 8.2. Protein solution (20 mg/mL DHODH with 2 mM DHO, 20.8 mM DDAO, and 400 µM inhibitor) was mixed 1:1 with mother liquor and hanging drops were used at room temperature. Crystals appeared after 48 hours and reached maximal size within one

week. Ligplot+ (Laskowski & Swindells, 2011; Wallace, Laskowski, & Thornton, 1995) was used to determine hydrophilic and hydrophobic interactions between inhibitors and DHODH molecules.

[0181] Immunoblot Analysis. PBS-washed cell pellets were resuspended in cold RIPA buffer supplemented with protease and phosphatase inhibitors. Protein lysates were normalized using BCA assay, diluted using RIPA and 4x laemmli loading dye, resolved on 4-12% Bis-Tris gels and electro-transferred to nitrocellulose membranes. After blocking with 5% nonfat milk in TBS + 0.1% Tween-20 (TBS-T), membranes were incubated overnight in primary antibodies diluted (per manufacturers instructions) in 5% BSA in TBS-T. Membranes were washed with TBST-T and incubated with HRP-linked secondary antibodies prepared at a 1:2500 dilution in 5% nonfat dry milk in TBS-T. HRP was activated by incubating membranes with a mixture of SuperSignal Pico and SuperSignal Femto ECL reagents (100:1 ratio). Exposure of autoradiography film was used for detection.

[0182] Statistical Analyses. Data are presented as mean ± SD with number of biological replicates indicated. Comparisons of two groups were calculated using indicated unpaired two-tailed Student's t-test and P values less than 0.05 were considered significant. Comparisons of more than two groups were calculated using one-way ANOVA followed by Bonferroni's multiple comparison tests, and P values less than 0.05/m, where m is the total number of possible comparisons, were considered significant.

[0183] References

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[0185] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in the application including, without limitation, patents, patent applications, articles, books, manuals, and treatises are hereby expressly incorporated by reference in their entirety for any purpose.

[0186] While various embodiments and aspects are shown and described herein, it will be obvious to those skilled in the art that such embodiments and aspects are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art. It should be understood that various alternatives to the embodiments described herein may be employed.

CLAIMS

What is claimed is:

1. A method for identifying a compound that inhibits cancer cell proliferation, the method comprising:

(i) contacting the compound with

(a) cancer cells and salvage pathway condition-specific growth media and de novo pathway condition-specific growth media; or

(b) cancer cells and salvage pathway condition-specific growth media; or

(c) cancer cells and de novo pathway condition-specific growth media;

wherein the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite;

(ii) identifying inhibition of cancer cell proliferation by the compound; thereby identifying the compound that inhibits cancer cell proliferation.

2. The method of claim 1, wherein (b) does not comprise de novo pathway condition-specific growth media; and wherein (c) does not comprise salvage pathway condition-specific growth media.

3. The method of claim 1, wherein:

(a) comprises cancer cells and pyrimidine nucleoside salvage pathway condition-specific growth media and de novo nucleotide pathway condition-specific growth media;

(b) comprises cancer cells and pyrimidine nucleoside salvage pathway condition-specific growth media; and

(c) comprises cancer cells and de novo nucleotide pathway condition-specific growth media.

4. The method of claim 1, wherein the method comprises contacting the compound with (a) and (b).

5. The method of claim 1, wherein the method comprises contacting the compound with (a) and (c).

6. The method of claim 1, wherein the method comprises contacting the compound with (b) and (c).

7. The method of claim 1, wherein the method comprises contacting the compound with (a), (b), and (c).

8. A method of treating cancer in a patient in need thereof, the method comprising:

(i) identifying the compound that inhibits cancer cell proliferation according to claim 1;

(ii) selecting the compound that inhibits caner cell proliferation in (b) or (c);

(iii) administering to the patient a therapeutically effective amount of the compound that inhibits caner cell proliferation in (b) or (c).

9. The method of claim 8, wherein the compound that inhibits caner cell proliferation in (b) does not inhibit cancer cell proliferation in (c).

10. The method of claim 8, comprising selecting the compound that inhibits caner cell proliferation in (b); and administering to the patient a therapeutically effective amount of the compound that inhibits caner cell proliferation in (b).

11. The method of claim 8, wherein the compound that inhibits caner cell proliferation in (b) is 3-[[4-(dimethylamino)-1-oxo-2-buten-1-yl]amino]-*N*-[3-methyl-4-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]benzamide, 4-[4-[[5-fluoro-4-[3-(prop-2-enoylamino)anilino]pyrimidin-2-yl]amino]phenoxy]-N-methylpyridine-2-carboxamide, or motesanib.

12. The method of claim 8, wherein the compound that inhibits caner cell proliferation in (c) does not inhibit cancer cell proliferation in (b).

13. The method of claim 8, comprising selecting the compound that inhibits caner cell proliferation in (c); and administering to the patient a therapeutically effective amount of the compound that inhibits caner cell proliferation in (c).

14. The method of claim 8, wherein compound that inhibits caner cell proliferation in (c) is 2-amino-*N*-[4-[5-phenanthren-2-yl-3-(trifluoromethyl)pyrazol-1-yl]phenyl] acetamide or N-(7-cyano-6-(4-fluoro-3-(2-(3-(trifluoromethyl)phenyl)acetamido)-phenoxy)benzo[d]thiazol-2-yl)cyclopropanecarboxamide.

15. The method of claim 8, wherein the cancer is pancreatic cancer.

16. The method of claim 15, wherein the pancreatic cancer is pancreatic ductal adenocarcinoma.

17. A method of treating pancreatic cancer in a patient in need thereof, the method comprising administering a therapeutically effective amount of a de novo nucleotide pathway inhibitor.

18. The method of claim 17, wherein the de novo nucleotide pathway inhibitor is a dihydroorotate dehydrogenase inhibitor.

19. The method of claim 17, wherein the de novo nucleotide pathway inhibitor is 2-amino-*N*-[4-[5-phenanthren-2-yl-3-(trifluoromethyl)pyrazol-1-yl]phenyl] acetamide or N-(7-cyano-6-(4-fluoro-3-(2-(3-(trifluoromethyl)phenyl)acetamido)-phenoxy)benzo[d]thiazol-2-yl)cyclopropanecarboxamide.

20. The method of claim 17, wherein the de novo nucleotide pathway inhibitor is not a pyrimidine nucleoside salvage pathway inhibitor.

21. A method of treating pancreatic cancer in a patient in need thereof, the method comprising administering a therapeutically effective amount of a pyrimidine nucleoside salvage pathway inhibitor.

22. The method of claim 21, wherein the pyrimidine nucleoside salvage pathway inhibitor is 3-[[4-(dimethylamino)-1-oxo-2-buten-1-yl]amino]-*N*-[3-methyl-4-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]benzamide, 4-[4-[[5-fluoro-4-[3-(prop-2-enoylamino)anilino]pyrimidin-2-yl]amino]phenoxy]-N-methylpyridine-2-carboxamide, or motesanib.

23. The method of claim 21, wherein the pyrimidine nucleoside salvage pathway inhibitor is not a de novo nucleotide pathway inhibitor.

24. The method of claim 17, wherein the pancreatic cancer is pancreatic ductal adenocarcinoma.

25. The method of claim 8, further comprising administering to the patient a therapeutically effective amount of an ATR kinase inhibitor.

26. The method of claim 25, wherein the ATR kinase inhibitor is berzosertib, 3-amino-6-(4-(methylsulfonyl)phenyl)-N-phenylpyrazine-2-carboxamide, ceralasertib, schisandrin B, 4-cyclohexylmethoxy-2,6-diamino-5-nitrosopyrimidine, dactolisib, 4[4-[(3R)3-methylmorpholin-4-yl]-6-[1-(methylsulfonyl)cyclopropyl]-pyrimidin-2-yl]-1H-indole, caffeine, wortmannin, or an analog of any one of the foregoing.

27. The method of claim 8, further comprising administering to the patient a therapeutically effective amount of an anti-cancer agent.

28. A composition comprising dihydroorotate dehydrogenase and N-(7-cyano-6-(4-fluoro-3-(2-(3-(trifluoromethyl)phenyl)acetamido)-phenoxy)benzo[d]thiazol-2-yl)cyclopropanecarboxamide.

29. The composition of claim 28, wherein N-(7-cyano-6-(4-fluoro-3-(2-(3-(trifluoromethyl)phenyl)acetamido)-phenoxy)benzo[d]thiazol-2-yl)cyclopropanecarboxamide and dihydroorotate dehydrogenase are bonded together via a hydrogen bond.

30. A composition comprising dihydroorotate dehydrogenase and 2-amino-*N*-[4-[5-phenanthren-2-yl-3-(trifluoromethyl)pyrazol-1-yl]phenyl] acetamide.

31. The composition of claim 30, wherein 2-amino-*N*-[4-[5-phenanthren-2-yl-3-(trifluoromethyl)pyrazol-1-yl]phenyl] acetamide and dihydroorotate dehydrogenase are bonded together via a hydrogen bond.

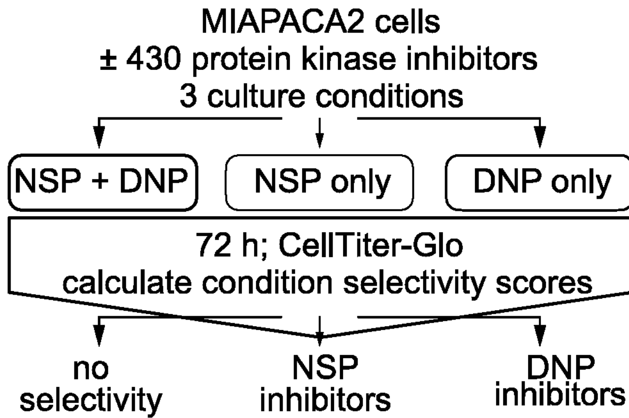


FIG. 1A

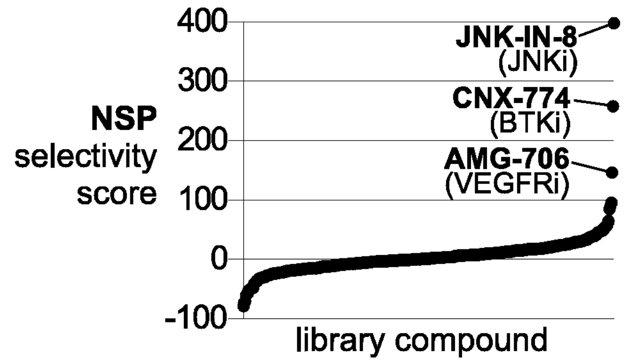


FIG. 1B

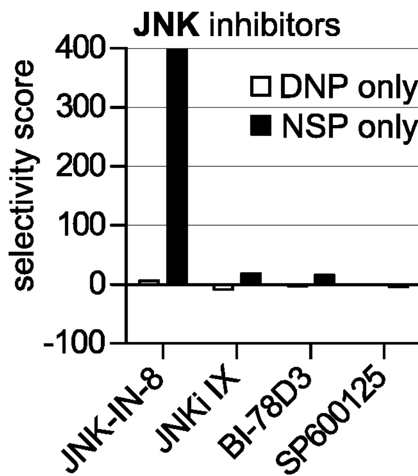


FIG. 1C

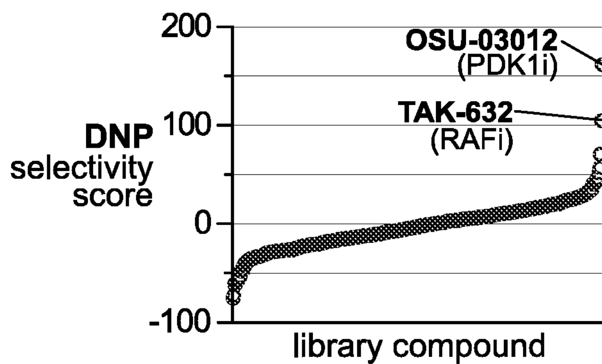


FIG. 1D

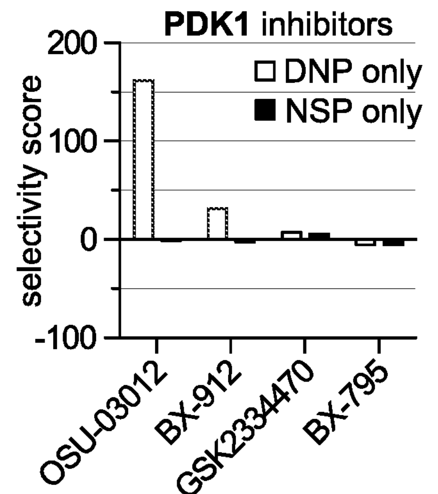


FIG. 1E

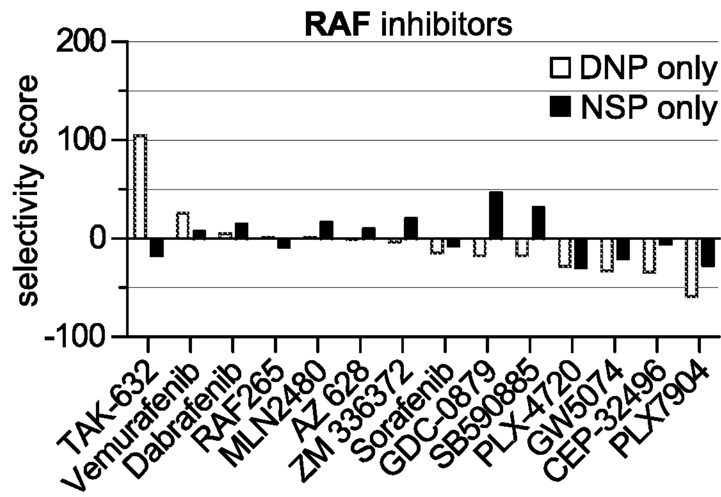


FIG. 1F

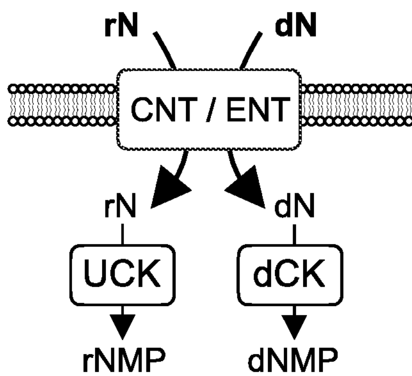


FIG. 2A

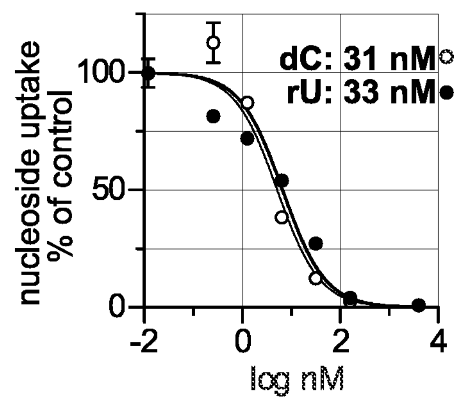


FIG. 2B

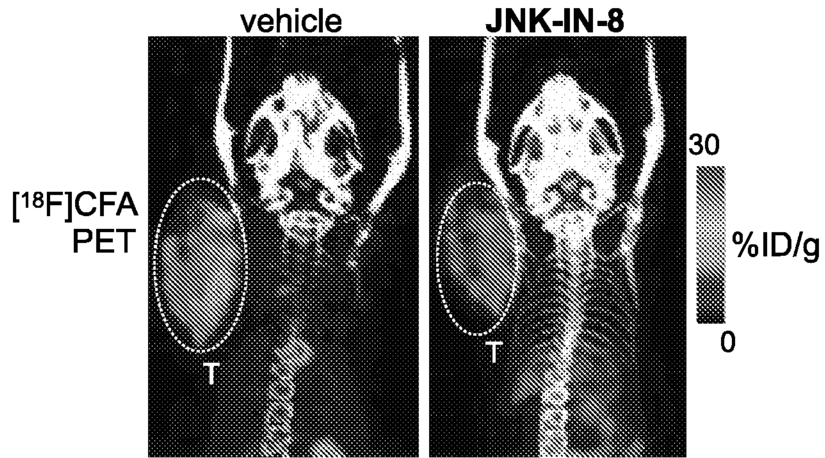


FIG. 2C

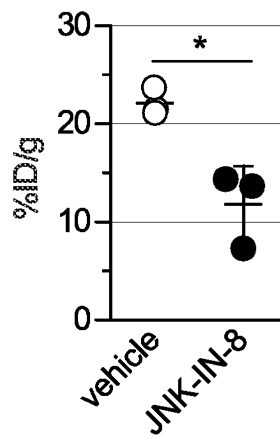


FIG. 2D

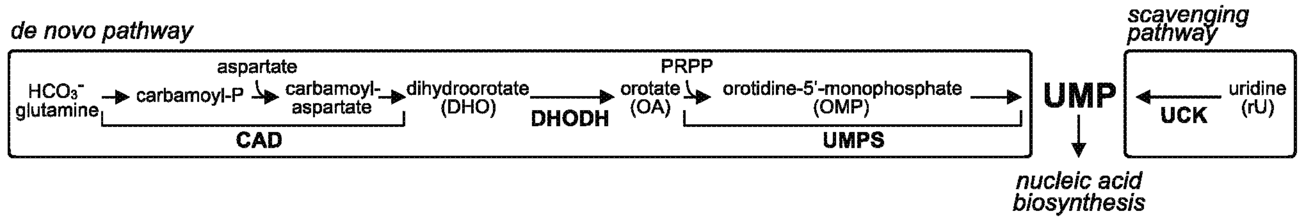


FIG. 3A

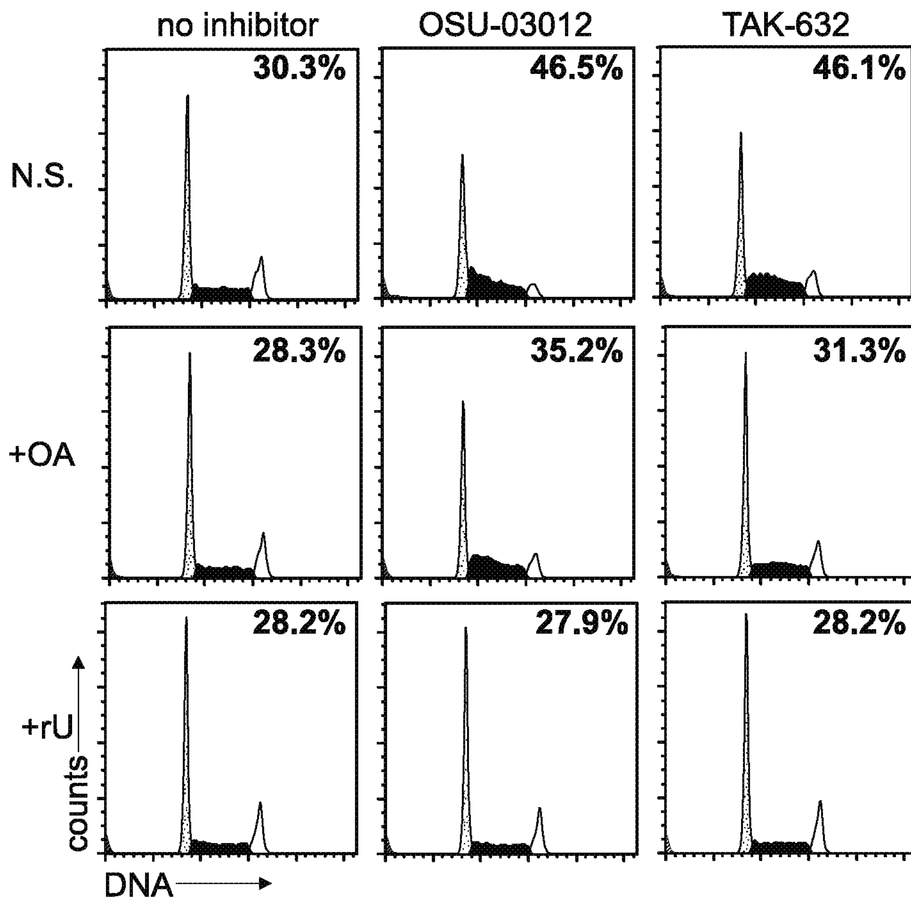


FIG. 3B

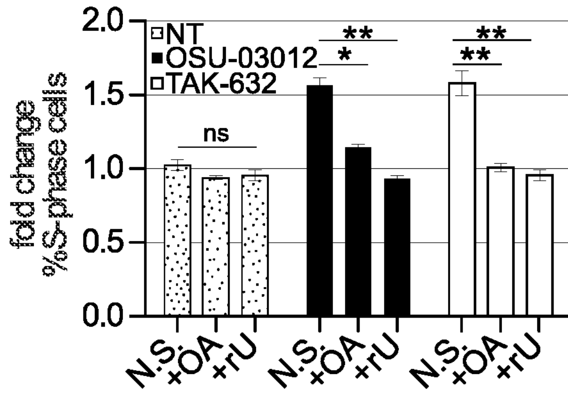


FIG. 3C

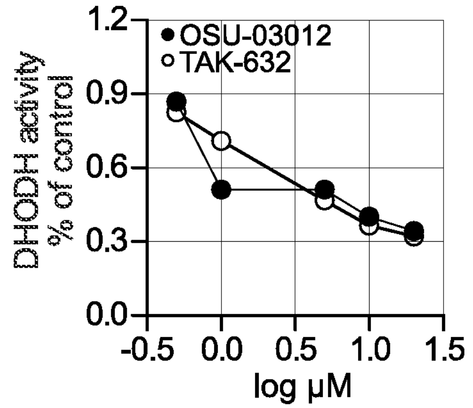
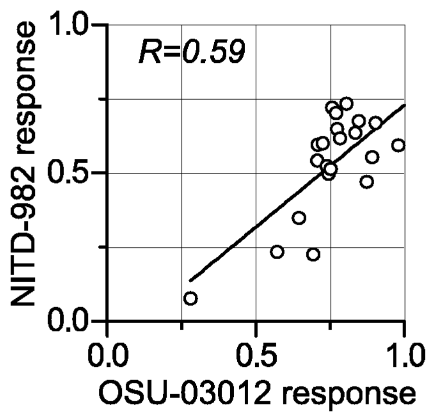


FIG. 3D



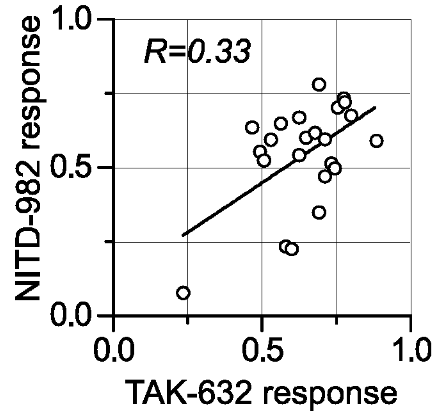


FIG. 3E

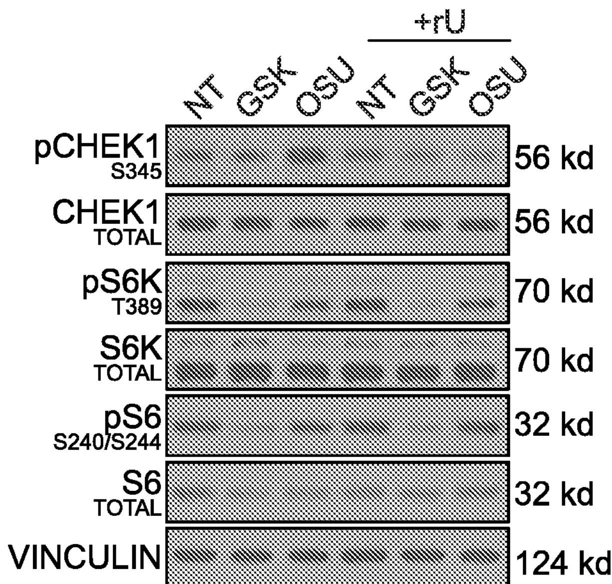


FIG. 3F

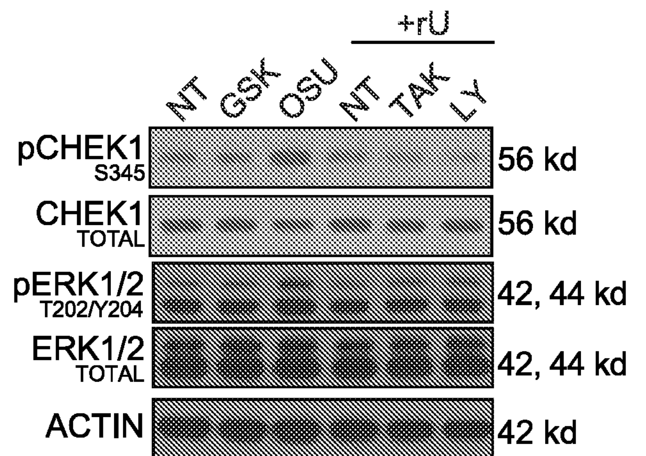


FIG. 3G

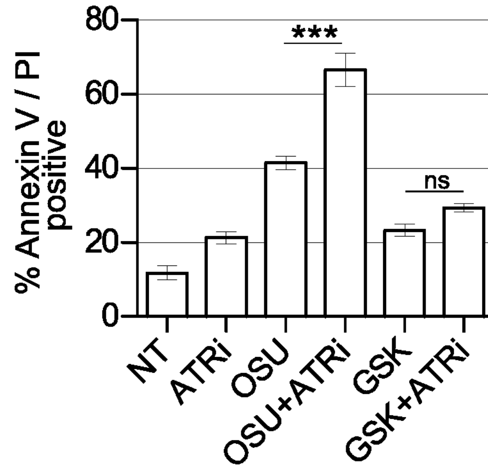


FIG. 3H

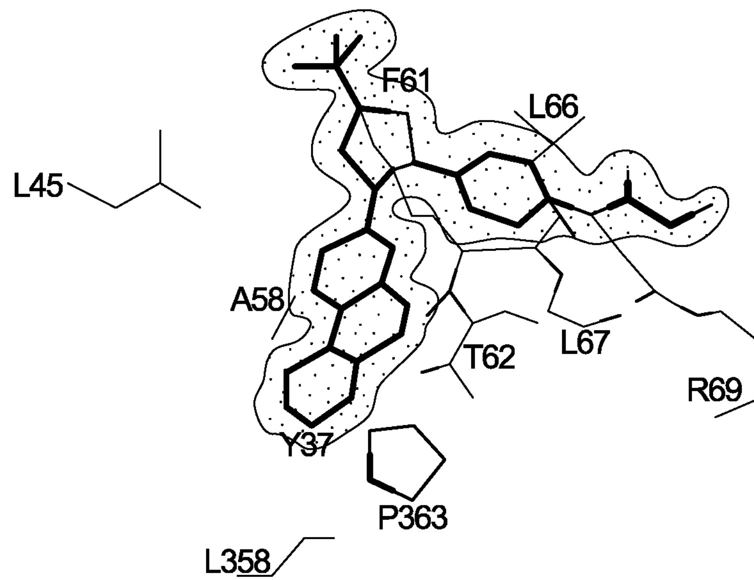


FIG. 4A

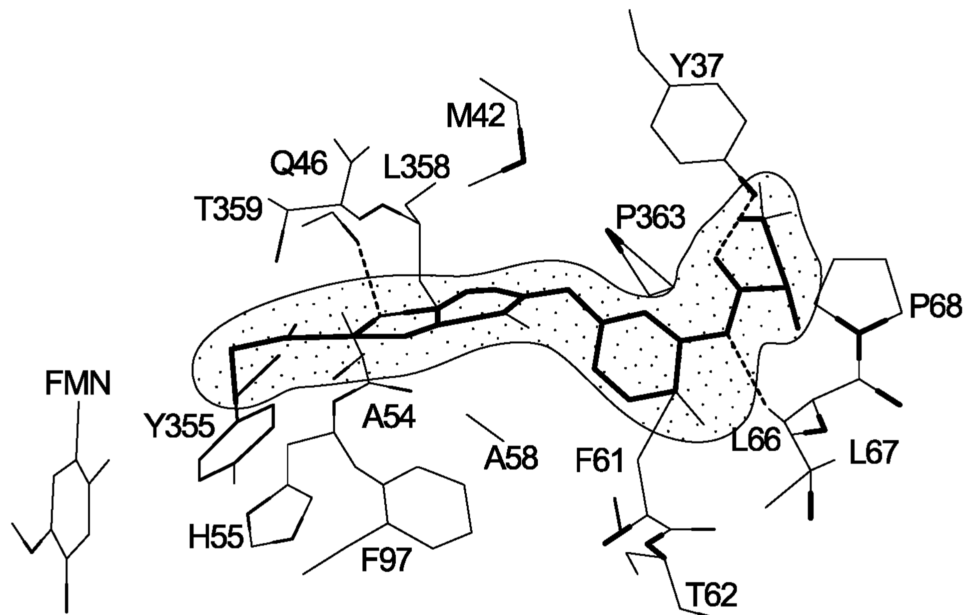


FIG. 4B

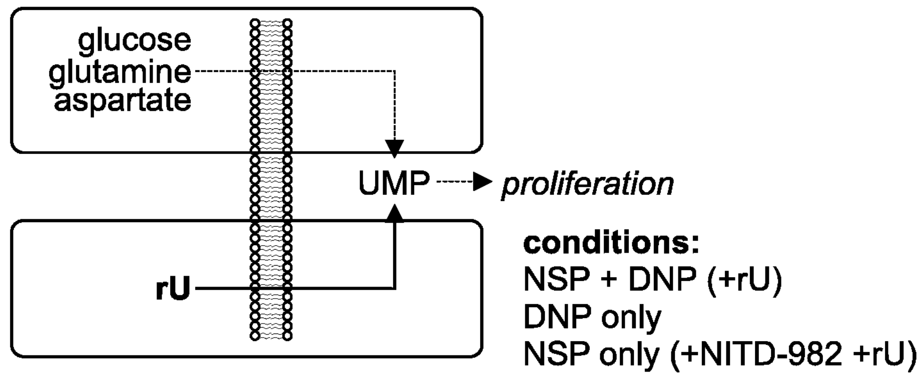


FIG. 5A

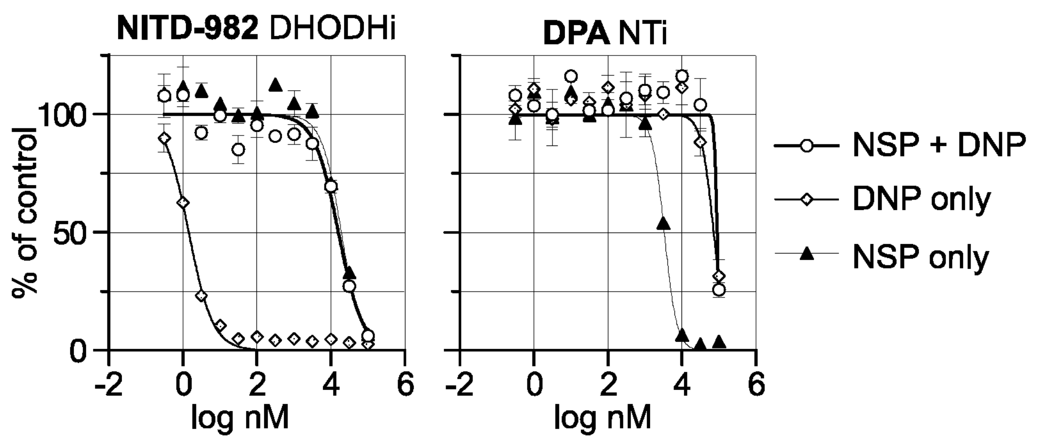


FIG. 5B

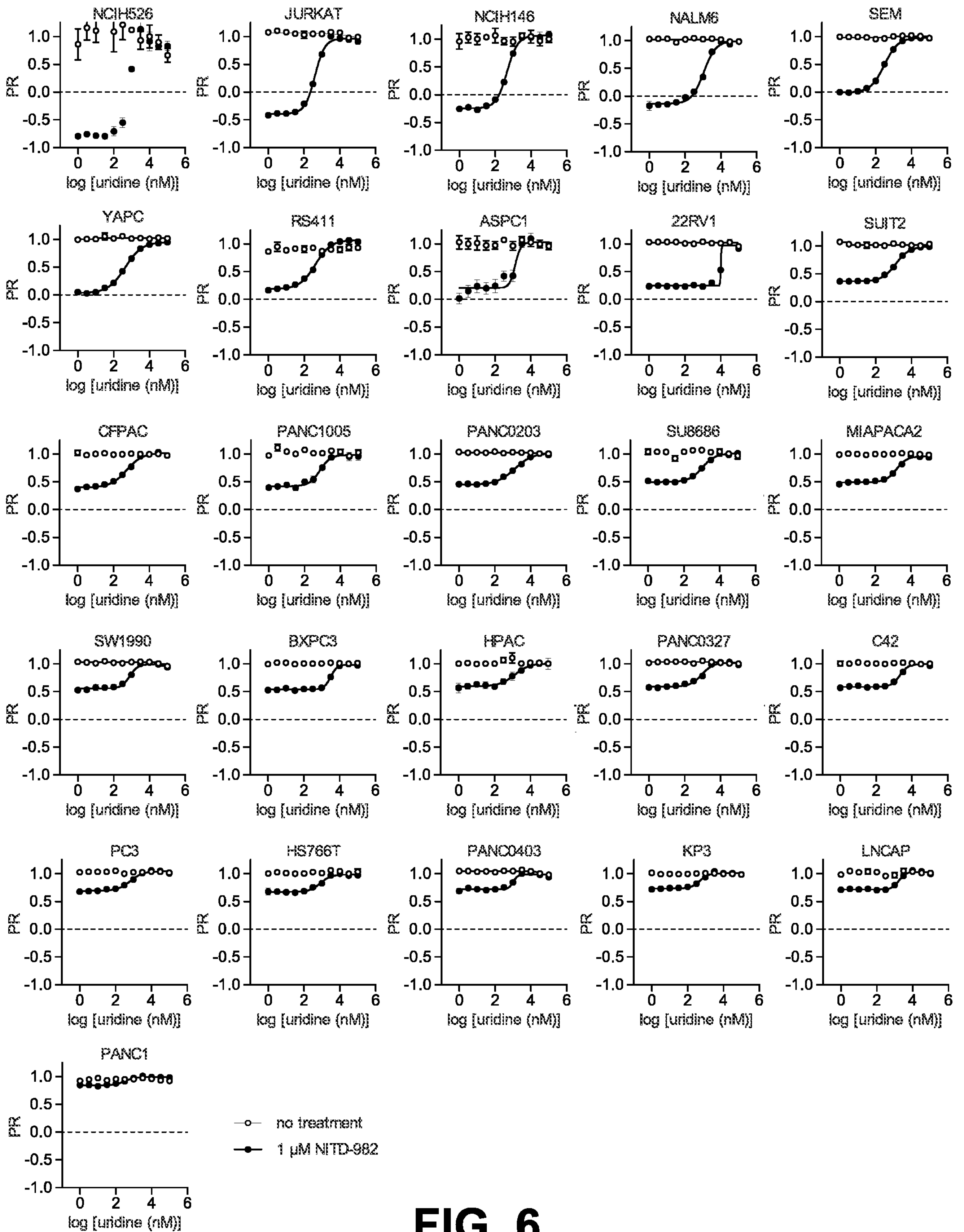


FIG. 6

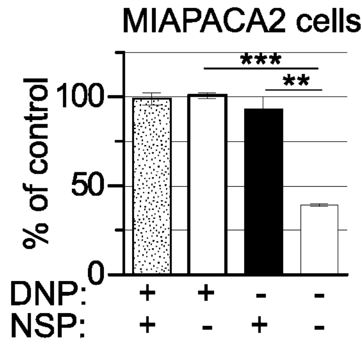


FIG. 7A

composite selectivity score

$$\sum_{n=1}^{7} (\%proliferation_{NSP+DNP} - \%proliferation_{DNP/NSP})_{C1} + \ldots$$

FIG. 7B

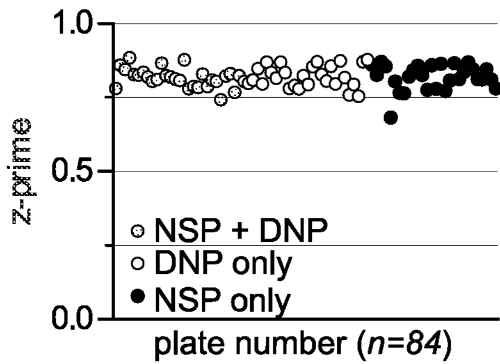


FIG. 7C

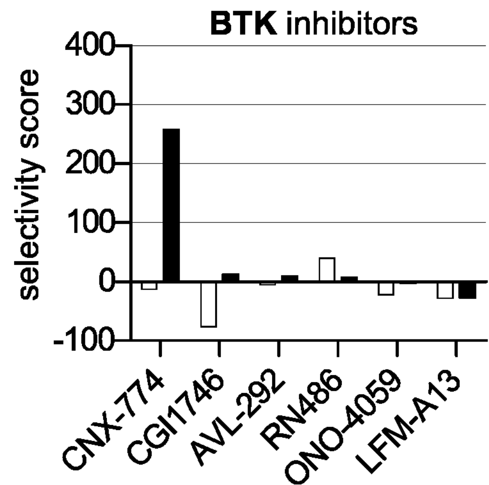


FIG. 7D

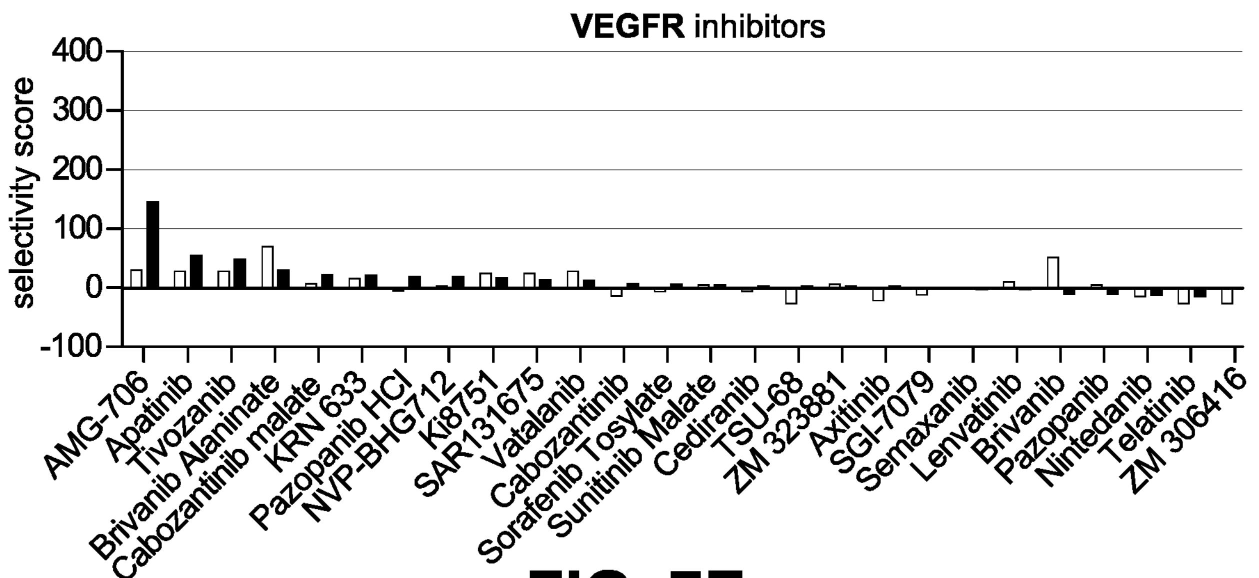


FIG. 7E

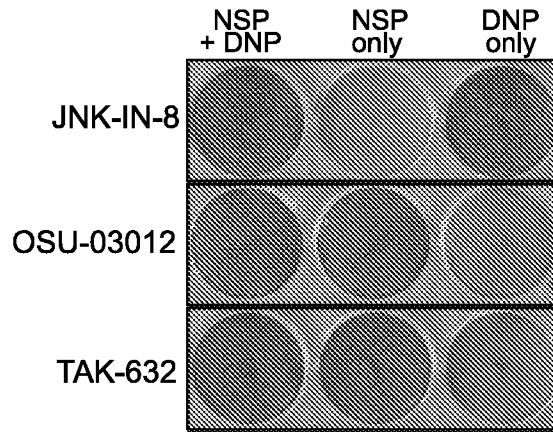


FIG. 7F

	JNK-IN-8	CNX-774	AMG-706
NSP+DNP IC_{50}	3090 nM	1700 nM	12441 nM
DNP IC_{50}	2990 nM	2270 nM	10381 nM
NSP IC_{50}	12.86 nM	76.7 nM	134 nM
dFdC EC_{50}	15.16 nM	36.2 nM	75.4 nM

FIG. 8A

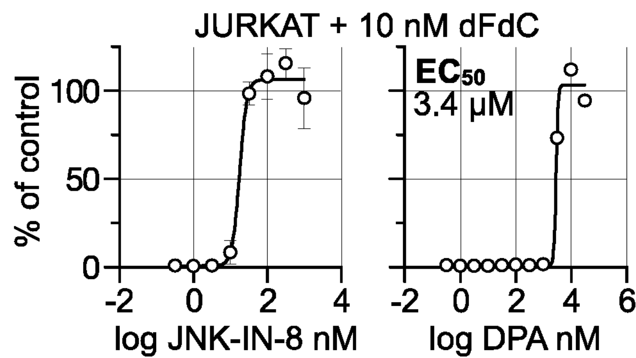


FIG. 8B

	OSU-03012	TAK-632
NSP+DNP IC_{50}	3829 nM	2346 nM
DNP IC_{50}	390.7 nM	579.8 nM
NSP IC_{50}	3426 nM	1978 nM

FIG. 8C

Structure	***DHODH + OSU-03012***	***DHODH + TAK-632***
PDB codes	6OC0	6OC1
Data collection statistics		
X-ray source and detector	LS-CAT 21-ID-D Dectris Eiger x 9M	LS-CAT 21-ID-G MARCCD 300
Wavelength (Å)	0.99987	0.97872
Temperature (K)	100	100
	1.40 (1.48-1.40)	2.7 (2.85-2.7)
Number of Reflections		
Observed	1,090,019 (113,565)	306,440 (48,502)
Unique	114,363 (17,418)	13,751 (2,169)
Completeness (%)	99.0 (94.2)	99.9 (99.8)
R_{meas} (%)	7.0 (54.5)	17.0 (183.3)
$CC_{1/2}$ (%)	99.9 (87.8)	99.9 (71.1)
Average I/σ(I)	19.2 (2.8)	21.6 (2.1)
Space group	P 3_2 2 1	P 2_1 3
Unit cell: a, b, c (Å)	90.01, 90.01, 123.07	113.67, 113.67, 113.67
Unit cell: α, β, γ (°)	90, 90, 120	90, 90, 90
Wilson B-factors ($Å^2$)	13.9	66.4
Refinement statistics		
Refinement program	REFMAC5	REFMAC5
R_{work} (%)	15.7	23.4
R_{free} (%)	19.1	29.1
Resolution range (Å)	48.55-1.40	46.45-2.70
Protein molecules per a.u.	1	1
Number of atoms:		
Protein	2845	2769
Water molecules	232	36
ORO + FMN	42	42
Inhibitor	34	39
R.m.s. deviation from ideal:		
Bond length (Å)	0.009	0.0021
Bond angles (°)	1.7114	1.264
Average B-factors ($Å^2$)		
Protein	23.9	73.0
Water molecules	32.6	44.7
ORO + FMN	12.2	56.6
Inhibitor	29.3	73.0
Ramachandran plot statistics (%)		
Most favored regions	97	86
Additionally allowed regions	3	14
Outlier regions	0	1

FIG. 9A

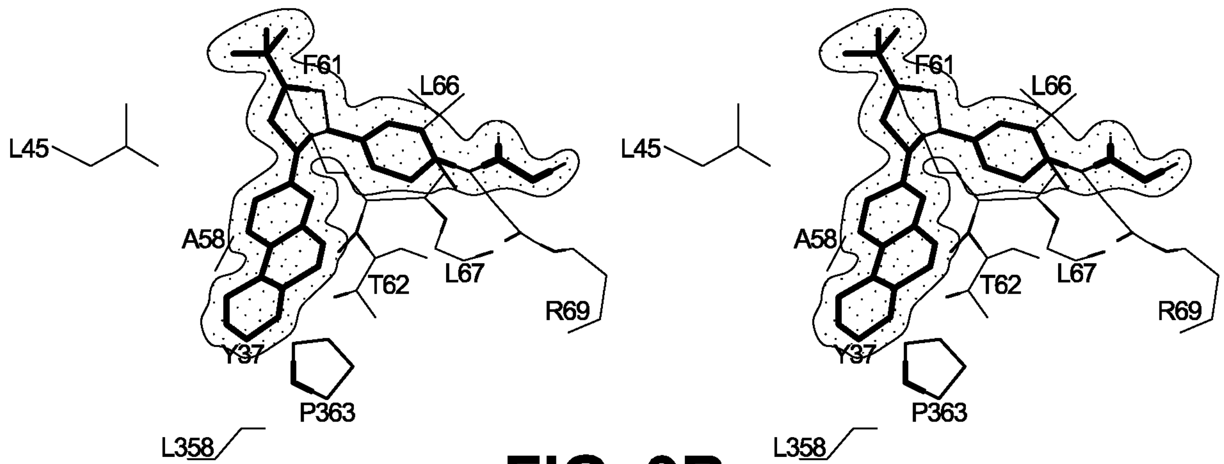


FIG. 9B

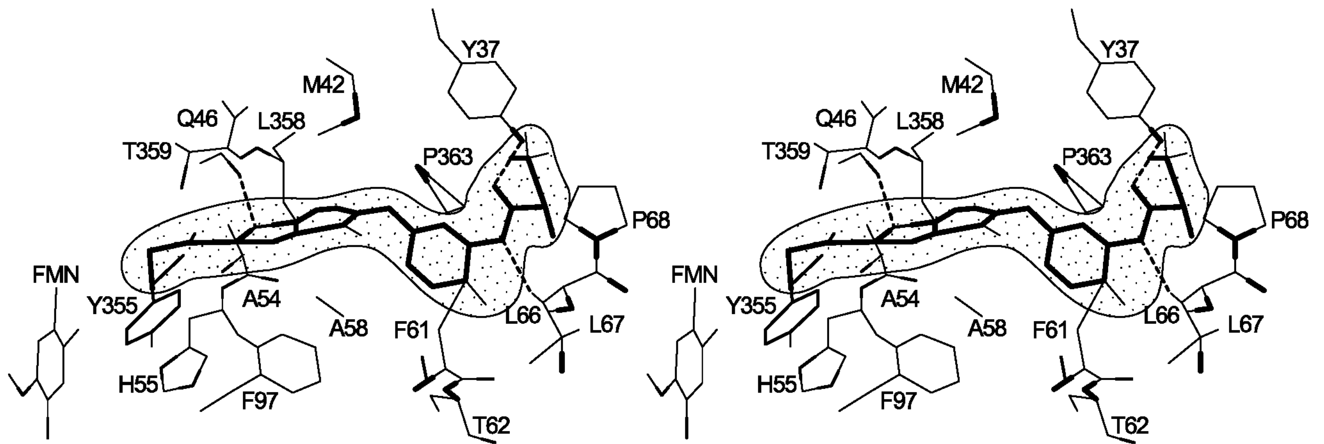


FIG. 9C

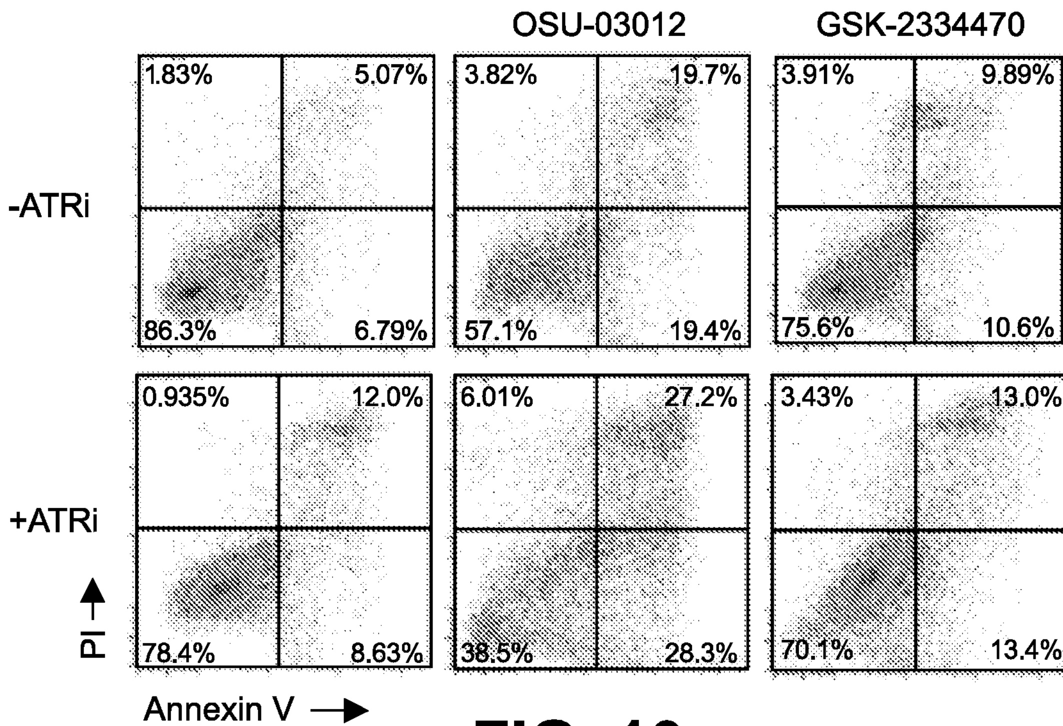


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20/33458

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 31/428, 31/5415; C07D 277/62 (2020.01)

CPC - A61K 31/428, 31/5415; C07D 277/62

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	(LE, TM et al.) 'ATR inhibition facilitates targeting of leukemia dependence on convergent nucleotide biosynthetic pathways'; 14 August 2017, Nature Communications; Volume 8, pages 1-14; abstract; page 2, first column, first paragraph; page 2, second column, third paragraph; page 3, first column, first paragraph; page 3, second column, first paragraph; page 3, figure 1; page 4, first column, first through third paragraphs; page 4, second column, first paragraph	1, 3
A	US 2010/0216810 A1 (OKANIWA, M et al.) 26 August 2010; paragraphs [0004], [0016], [0050]-[0051], [0466], [0504], [0820]	1, 3
A	US 2016/0067321 A1 (TRUSTEES OF DARTMOUTH COLLEGE) 10 March 2016; paragraphs [0003], [0006], [0026]-[0027], [0064]	1, 3
A	WO 2016/130562 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 18 August 2016; entire document	1, 3
A	(LEWIS, TA et al.) 'Development of ML390: A Human DHODH Inhibitor That Induces Differentiation in Acute Myeloid Leukemia'; 28 September 2018, ACS Medicinal Chemistry Letters; Volume 7, Issue 12, pages 1112-1117; DOI: 10.1021/acsmedchemlett.6b00316	1, 3
A	US 2009/0111799 A1 (CHEN, CS et al.) 30 April 2009; entire document	1, 3
T, X	(ROSSER, E) 'Identification, Development, and Evaluation of Small-Molecule Modulators of Nucleotide Metabolism' 2020, UCLA Electronic Theses and Dissertations <https://escholarship.org/uc/item/8kn6r3nx>; entire document	1, 3

☐ 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
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 2020 (04.09.2020)	20 OCT 2020

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20/33458

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.:
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:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-16, 25-27 and cancer cells and salvage pathway condition-specific growth media and de novo pathway condition-specific growth media (media) are directed to methots of identifying a compound, and methods of treatment associated therewith.

-***-Continued on supplemental page-***-

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.:
1, 3; cancer cells and salvage pathway condition-specific growth media and de novo pathway condition-specific growth media (media)

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20/33458

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

The methods will be searched to the extent they encompass cancer cells and salvage pathway condition-specific growth media and de novo pathway condition-specific growth media (first exemplary media). Applicant is invited to elect additional media, with fully specified elements thereof (e.g. no optional or variable elements) for each, to be searched. Additional media will be searched upon the payment of additional fees. It is believed that claims 1 (in-part) and 3 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass a method of contacting the compound with cancer cells and salvage pathway condition-specific growth media and de novo pathway condition-specific growth media (method). Applicants must specify the claims that encompass any additionally elected method(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a method for identifying a compound that inhibits cancer cell proliferation, the method comprising: contacting the compound with cancer cells and salvage pathway condition-specific growth media (method).

Group II, Claims 17-20, 24 are directed toward a method of treating pancreatic cancer in a patient in need thereof, the method comprising administering a therapeutically effective amount of a de novo nucleotide pathway inhibitor.

Group III, Claims 21-23 are directed toward a method of treating pancreatic cancer in a patient in need thereof, the method comprising administering a therapeutically effective amount of a pyrimidine nucleoside salvage pathway inhibitor.

Group IV, Claims 28-29 are directed toward a composition comprising dihydroorotate dehydrogenase and N-(7-cyano-6-(4-fluoro-3-(2-(3-(trifluorornethyl)phenyl)acetamido)-phenoxy)benzo[dithiazol-2-yl)cyclopropanecarboxamide.

Group IV, Claims 30-31 are directed toward a composition comprising dihydroorotate dehydrogenase and 2-amino-N-[4-[5-phenanthren-2-yl-3-(trifluoromethyl)pyrazol-1-yl]phenyl]acetamide.

The inventions listed as Groups I+ and II-V do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Groups I+ include salvage pathway condition-specific growth media and de novo pathway condition-specific growth media, not present in any other Groups; the special technical features of Group II include a de novo nucleotide pathway inhibitor, not present in any other Groups; the special technical features of Group III include a pyrimidine nucleoside salvage pathway inhibitor, not present in any other Groups; the special technical features of Group IV include N-(7-cyano-6-(4-fluoro-3-(2-(3-(trifluorornethyl)phenyl)acetamido)-phenoxy) benzo[dithiazol-2-yl)cyclopropanecarboxamide, not present in any other Groups; and the special technical features of Group V include 2-amino-N-[4-[5-phenanthren-2-yl-3-(trifluoromethyl)pyrazol-1-yl]phenyl]acetamide, not present in any other Groups.

There is no single technical feature that is shared by all of the above Groups.

Groups I+, II and III share the technical features including: a method of treating cancer in a patient in need thereof, the method comprising administering a therapeutically effective amount of a compound. Groups II and III further share the technical features including: pancreatic cancer; and a pathway inhibitor. Groups IV and V share the technical features including: a composition comrprising a compound.

However, these shared technical features are previously disclosed by US 2014/0219961 A1 to Jung et al. (hereinafter 'Jung').

Jung discloses a method of treating cancer in a patient in need thereof (a method of treating cancer in a patient in need thereof; Claim 1), the method comprising administering a therapeutically effective amount of a compound (the method comprising administering a therapeutically effective amount of a compound; Claim 1); pancreatic cancer (pancreatic cancer; paragraph [0058]); and a pathway inhibitor (a pathway inhibitor; paragraph [0059]); and a composition comprising a compound (a composition comprising a compound; abstract).

Groups I+ share the technical features including: a method for identifying a compound that inhibits cancer cell proliferation, the method comprising: (i) contacting the compound with (a) cancer cells and salvage pathway condition-specific growth media and de novo pathway condition-specific growth media; or (b) cancer cells and salvage pathway condition-specific growth media; or (c) cancer cells and de novo pathway condition-specific growth media: wherein the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite; (ii) identifying inhibition of cancer cell proliferation by the compound; thereby identifying the compound that inhibits cancer cell proliferation.

However, these shared technical features are previously disclosed by the article entitled "ATR inhibition facilitates targeting of leukemia dependence on convergent nucleotide biosynthetic pathways" by Le, et al. (hereinafter 'Le').

Le discloses a method for identifying a compound that inhibits cancer cell proliferation (method of identifying if DI-82 and/or VE-822 (compounds) inhibit cancer; abstract; page 2, first column, first paragraph; page 2, second column, third paragraph), the method comprising: (i) contacting the compound with (a) cancer cells and salvage pathway condition-specific growth media and de novo pathway condition-specific growth media, wherein the salvage pathway and de novo pathway are convergent metabolic pathways producing a common metabolite (method comprises releasing (contacting) cancer cells into media (growth media) containing VE-822 and DI-82 (compounds) and the method measures the levels of de novo and salvage biosynthesis (media is condition specific for de novo and salvage pathways); page 2, second column, third and fourth paragraphs; page 3, first column, first paragraph; page 3, second column, first paragraph; page 3, figure 1; page 4, first column, first paragraph); (ii) identifying inhibition of cancer cell proliferation by the compound; thereby identifying the compound that inhibits cancer cell proliferation (VE-822, an inhibitor of ATR, along with DI-82, a dCK inhibitor, inhibited cancer cell proliferation, which would identify DI-82 and/or VE-822 (compounds) as compounds that inhibit cancer cell proliferation); abstract; page 4, first column, first through third paragraphs; page 4, second column, first paragraph).

Since none of the special technical features of the Groups I+ and II-V inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Jung and Le references, unity of invention is lacking.